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THE PREVENTION OF SIMPLE GOITER IN MAN *

SECOND PAPER

O. P. KIMBALL, M.D., AND DAVID MARINE, M.D.
CLEVELAND

In Article I¹ we gave a brief review of the experimental work on which the assertion is based that simple goiter is probably the easiest of all known diseases to prevent. We gave a survey of the incidence and types of thyroid enlargement in the schoolgirls of Akron (Ohio) from the fifth to the twelfth grades, inclusive, and the plan of prevention used.

The plan in operation was arranged from the standpoint of simplicity, practicability, economy and the possible scientific value of the data obtained. First, a census of the condition of the thyroid gland was taken of all girls between the fifth and twelfth grades, inclusive, and the findings recorded on individual cards. This card will be used throughout the whole series of observations, the condition of the thyroid noted each year and a record of all treatment kept on the back of the card.

No.	Date
Name	School
Age	Weight
Grade	Physical Development
Tonsils-Adenoids	Class Standing
Thyroid	1
Simple	2
Adenomas	3
Thyroid-tract	4
Duration
Remarks

It was planned that the thyroid examinations should be made by a single examiner in order to make the standard used constant, and the data obtained uniform. At the time of the first examination, however, it was clearly foreseen that Dr. Marine would be called to military duty. Therefore, in order that the junior author should be trained to examine and classify the cases in the November examination as nearly as possible precisely in the same way as was done in the April examination, the April examination was made by both authors conjointly. It is obvious that much of the value of observations of this kind must depend on the uniformity of the methods and classification.

*From The H. K. Cushing Laboratory of Experimental Medicine, Western Reserve University.

1. The Prevention of Simple Goiter in Man, Jour. Lab. and Clin. Med., 1917, 3, 40.

The thyroid glands were examined from the standpoint of Normals, Slight, Moderate and Marked Enlargements, Adenomas, Persistent Thyroglossal Tracts, and the girls for gross manifestations of myxedema, or exophthalmic goiter. No obvious case of either myxedema or exophthalmic goiter has been found.

Under *Normal* we have included all glands (a) which are not visible as a bulging of the skin across the trachea; (b) having a barely detectable band of thyroid tissue across the trachea on palpation, and (c) absence of a well defined thyroglossal stalk (so-called pyramidal process).

The cases with *Enlarged Thyroids* have been divided into three arbitrary groups: (1) Slight, (2) Moderate and (3) Marked Enlargement. Under *Slight Enlargement* we have grouped those cases with (a) visible bulging of the skin over the thyroid isthmus (except in very stout children), and (b) a widened and thickened isthmial band or mass on palpation. If the isthmus can not be seen or felt, it can be felt by having the child swallow while the finger or thumb is held against the trachea just below the cricoid cartilage. Under *Moderate Enlargement* we have grouped those with gross deformity, bulging of the neck laterally from the enlarged lobes and marked bulging of the skin anteriorly from the enlarged isthmus. Under *Marked Enlargement* we have grouped those cases with excessive deformity.

Analysis of Thyroid Examinations.—In April, 1917, 3,872 girls of the fifth to twelfth grades, inclusive, were examined and the general results are given in Table 1. In November, 4,415 girls of the fifth to twelfth grades, inclusive, were examined and the new records are given in Table 1. The new records each year will be classed in this table.

TABLE 1.—CONDITION OF THYROID GLAND

	Normal		Slight Enlargements		Moderate Enlargements		Marked Enlargements		Adenomas	
	Pupils	%	Pupils	%	Pupils	%	Pupils	%	Pupils	%
April, 1917	1,688	43.59	1,931	49.88	246	6.35	7	0.18	29	1.01
Nov., 1917*	831	47.00	820	46.20	121†	6.80				

* Fifth grade girls and girls who entered the Akron schools since April, 1917.

† This number is greater than it would be if only fifth grade pupils were examined; two large schools, one a high school, accidentally destroyed all records of those girls not taking treatment, and therefore had to be recorded as new records.

For the prophylactic treatment we selected sodium iodid on the grounds of economy and ease of administration. Regarding the amounts that should be given, we had no data except from animal experimentation, and as we have pointed out repeatedly, exceedingly small amounts of iodine are effective. In all our dispensary work with children we have used either syrup of hydriodic acid or syrup of ferrous iodid, in 1 c.c. doses daily for two or three weeks, repeated twice yearly. The dosage is much less than the therapeutic dose of

iodids in other instances in which they are so extensively used, and there can be no reasonable doubt that the action is very different.

We, therefore, arbitrarily selected to use 2 gm. sodium iodid, given in 0.2 gm. doses each school day, for each pupil in the fifth, sixth, seventh and eighth grades; and 4 gm. in 0.4 gm. doses each school day for each pupil in the ninth, tenth, eleventh and twelfth grades. These amounts were given in May, 1917, but in November, 1917, we gave 2 gm. to each pupil from the fifth to twelfth grades, inclusive, since this amount in the year's use gave such definite results. As was pointed out in the previous paper, it was thought likely that the dose would be materially reduced. This amount (2 gm.) will be given again in April, 1918. The treatment is given at the school by the teacher or principal and the number of doses recorded. A record is kept, both of those who take the treatment and of those who do not, and all pupils are to be examined annually and the thyroid conditions recorded.

A complete reexamination of all girls from the fifth to twelfth grades was made in November, 1917. There were 1,772 new records (Table 1). These include (a) this year's *fifth* grade; (b) pupils of all grades above the fifth entering Akron schools since April, 1917, and (c) all the girls in two schools (*not* taking treatment) whose records had been accidentally destroyed (see footnote, Table 1). All those previously examined were classed either as taking prophylactic treatment or not taking prophylactic treatment. The results are summarized in Table 2.

TABLE 2.—SUMMARY

Pupils Taking Prophylactic Treatment	Pupils	%	Pupils Not Taking Prophylactic Treatment	
			Pupils	%
Thyroids remained normal	283	100.00	637	74.0
Increased from normal to slight goiter	0	0.0	259	26.0
Small goiters (unaltered)	287	66.0	759	87.0
Small goiters (disappeared)	141	33.5	10	1.2
Small goiters (increased)	2	0.5	103	11.8
Large goiters (unaltered)	34	66.7	106	95.5
Large goiters (decreased)	17	33.3	5	4.5
Total	764		1,879	
Total number of girls examined	4,415			

It will be seen that not a single pupil in whom the thyroid was normal last year and who took iodine, showed any enlargement, while of those not taking iodine, 26 per cent. showed definitely enlarged thyroids—some moderately large goiters. Even more than a prophylactic action is shown in the results—just one third of the goiters marked “small goiters” disappeared; and one third of those marked “moderate goiters” showed a decrease of 2 cm. or more. Accordingly, a distinct therapeutic effect is clearly demonstrated.

It was suggested by some physicians that we would have many cases of iodine rash. We spoke of this possibility in every school and asked the principal and teachers to look for symptoms and call the

attention of the school nurse and physician to every possible case. There were more than a thousand girls who took the full treatment, and only five developed any noticeable rash. None of these gave any trouble and the condition lasted only three or four days. Four of the girls continued the treatment and paid no attention to it, while the fifth asked to be excused from further treatment and the rash promptly cleared up.

As to the possibility of producing symptoms of Basedow's disease by giving iodine (in small doses) to a large number of girls indiscriminately, we can say that we have not seen a single instance in which any sign of such an effect was produced.

The earnestness with which the school girls have taken up the prophylaxis of goiter is encouraging for the practical application of this work. It is entirely elective on the part of the girls, and last year 1,080 girls finished the treatment. This year approximately 2,000 girls are taking the treatment.

SUMMARY

1. Simple goiter can be prevented by the administration of small amounts of iodine.
2. One third of the cases of uncomplicated simple goiter disappear or are markedly decreased by the use of a small amount of iodine, given internally.
3. There is no danger of producing a toxic condition ("Basedow's disease").
4. A very small proportion of the cases (at most 0.5 per cent.) may develop an iodine rash, which promptly clears up on stopping the treatment.

We wish to thank Prof. H. V. Hotchkiss (Superintendent of the Public Schools), the Board of Education and the Principals of the several schools; through whose excellent organization and system, as well as the interest in the problem, the work was made possible.

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THE PREVENTION OF SIMPLE GOITER IN MAN *

THIRD PAPER

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AND

DAVID MARINE, M.D.

CLEVELAND

This report is based on the reexamination of the girls in the public schools of Akron, Ohio, in grades from the fifth to the twelfth, inclusive, made from Nov. 26 to Dec. 3, 1918—nineteen months after beginning the prophylactic use of iodine. The first report¹—a survey of the incidence of thyroid enlargements (goiter)—was based on the examination made in April, 1917. The second report² gave the results of the examination in November, 1917—seven months after beginning the prophylactic use of iodine.

The same classification of the condition of the thyroid has been used as in previous examinations, namely: normal, slight, moderate and marked enlargements, adenomas and persistent thyroglossal tracts.³ The pupils were further examined for gross manifestations of exophthalmic goiter and myxedema. No obvious case of either of these diseases was detected.

ANALYSIS OF THE RECORDS OF NEW PUPILS

The results of these examinations are given in Table 1.

* From the H. K. Cushing Laboratory of Experimental Medicine, Western Reserve University.

* Aided by a Grant from the Committee on Therapeutic Research of the Council on Pharmacy and Chemistry of the American Medical Association.

1. Marine, David, and Kimball, O. P.: Prevention of Goiter in Man, *J. Lab. & Clin. M.* **3**: 40-43 (Oct.) 1917.

2. Kimball, O. P., and Marine, David: Prevention of Goiter in Man, Second Paper, *Arch. Int. Med.* **22**: 41-44 (July) 1918.

3. Persistence of the thyroid tract is the best evidence of enlargement of the thyroid during fetal life.

For comparison and reference, the figures of the original survey in April, 1917, and of the second examination in November, 1917, are added. The subjects included in this table are all new admissions to the public schools and presumably had not previously received iodine. Owing, however, to the very extensive use of iodine in some form, both by the public and the profession, it is probable that some of these pupils had had iodine for one reason or another, but no attempt was made to detect such cases. Iodine administered in any form markedly affects the thyroid.

The figures in the first line represent the results of the original survey of all girls in grades from the fifth to the twelfth, inclusive. The figures in the second line represent: (1) incoming fifth grade girls; (2) girls entering other grades of the public schools between April and November, 1917, and (3) girls of two

TABLE 1.—ANALYSIS OF THE RECORDS OF NEW PUPILS

Date Exam-ined	Total Exam-ined	Total New Cases	Normal Pupils		Enlargements						Adenomas		
			No.	%	Slight		Moderate		Marked			No.	%
					No.	%	No.	%	No.	%			
April, 1917	3,872	3,872	1,688	43.5	1,931	49.9	246	6.3	7	0.2	39	1.0	
Nov., 1917	4,415	1,772	831	47.0	820	46.2	121	6.8					
Nov., 1918	4,277	1,873	1,087	55.4	779	41.6	53	2.8	4	0.2	6	0.3	

schools that accidentally lost the records of those not taking the prophylactic treatment. The figures in the third line represent only girls in the incoming fifth grade and girls entering other grades.

The progressive increase in the percentage of normal thyroids (43.6, 47 and 55.4 per cent.) for the three periods is due to the preponderance of fifth grade girls in the second and third groups. Fifth grade girls average 10 years of age and are for the most part below the age of the greatly increased incidence of thyroid enlargement.

EFFECT OF PROPHYLACTIC TREATMENT

The same method as outlined in the first paper and modified in the second paper was used, i. e., 2 gm. of sodium iodide were given in 0.2 gm. doses for ten consecutive school days, repeated each autumn and spring.

TABLE 2.—RECORDS OF PUPILS TAKING PROPHYLACTIC TREATMENT

Date	Total Number	Thyroids Remaining Normal		Thyroids Enlarged from Normal to Slightly Enlarged		Slightly Enlarged			Moderately and Markedly Enlarged		
		No.	%	No.	%	Unaltered		Increased	Unaltered		Decreased
						No.	%		No.	%	
November, 1917.....	764	283	37.0	0	0.0	287	37.6	141	18.4	34	4.4
November, 1918.....	1,121	469	41.8	0	0.0	354	31.6	218	19.4	29	2.6
								0	0	0.0	51

TABLE 3.—RECORDS OF PUPILS NOT TAKING PROPHYLACTIC TREATMENT

Date	Total Number	Thyroids Remaining Normal		Thyroids Enlarged from Normal to Slightly Enlarged		Slightly Enlarged			Moderately and Markedly Enlarged		
		No.	%	No.	%	Unaltered		Increased	Unaltered		Decreased
						No.	%		No.	%	
November, 1917.....	1,879	637	33.9	259	13.8	759	40.4	10	0.5	106	5.6
November, 1918.....	1,283	496	38.7	94	7.3	424	33.1	170	13.2	52	4.1
								17	1.3	80	2.3

The results are given in Tables 2 and 3. For reference and comparison, the figures for the November, 1917, examination are added.

The most striking fact brought out is that not a pupil in whom the thyroid was normal at the November, 1917, examination, and who took iodine, showed any thyroid enlargement; while of those not taking iodine, 15.9 per cent. showed definite enlargement. This effect is similar to that noted in last year's examination. As was noted last year, a distinct therapeutic effect is again observed in that the glands of 38.1 per cent. of the pupils with slightly enlarged glands decreased following the use of iodine, while of the glands of those listed as not taking iodine 27.8 per cent. showed a decrease in size. This difference is much less than that found last year and suggests that many pupils with slight goiter were taking iodine privately. The same therapeutic effect is also noted in those with moderate and marked enlargements, and again the percentage differences between those taking and those listed as not taking iodine is less than last year's figures.

The main effects of the administration of iodine observed during the second year are similar to those noted during the first year. The danger of iodism or of exophthalmic goiter from the use of such amounts of iodine as were given is shown to be negligible.

SUMMARY

1. Simple goiter in man may be prevented on a large scale.
2. The method used is practical and economical, and can be recommended as a public health measure in goiter districts.
3. Two gm. of sodium iodide given twice yearly, as we have indicated, seems adequate.

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American Medical Association, 535 N. Dearborn St., Chicago*

PREVENTION OF SIMPLE GOITER IN MAN

FOURTH PAPER *

DAVID MARINE AND O. P. KIMBALL

CLEVELAND

In previous publications ¹ we have outlined the plan of prevention, presented the data of the incidence of thyroid enlargements as determined by annual surveys of all new pupils in the Akron public schools, and the results of the prophylactic use of sodium iodid for nineteen months. The present paper deals with the data obtained at the fourth general examination made October 13-17, 1919, together with summaries and conclusions based on observations extending over a period of thirty months.

ANALYSIS OF THE RECORDS OF NEW PUPILS

The general data of the clinical condition of the thyroid gland are given in Table 1. For comparison and reference the figures for the three previous examinations are also given.

The pupils included in this table are new admissions to all grades from the fifth to the twelfth, inclusive, and presumably had not previously received iodin. The figures in the first line represent the results of the original survey of all girls in grades from the fifth to the twelfth, inclusive. The figures in the second line include (1) incoming fifth grade girls, (2) girls entering grades above the fifth grade, and (3) girls of two schools that accidentally lost the records of those not taking the treatment. The figures in the third and fourth lines include (1) incoming fifth grade girls and (2) girls entering grades above the fifth. The progressive increase in the percentage of normal thyroids (43.6, 47.0, 55.4 and 65.4) and the corresponding progressive decrease in the percentage of enlarged thyroids whether taken together (56.4, 53.0, 44.6 and 34.5) or as separate groups (slightly enlarged, moderately enlarged and markedly enlarged) are due to the increasing preponderance of fifth grade girls in the second, third and fourth groups. This is also shown in Table 2, where all new pupils are grouped according to ages. Fifth grade pupils average from 10 to 11 years of age, and approxi-

* From the Department of Experimental Medicine, Western Reserve University.

¹ Aided by a Grant from the Committee on Therapeutic Research of the Council on Pharmacy and Chemistry of the American Medical Association.

1. Marine, D., and Kimball, O. P.: The Prevention of Simple Goiter in Man, *J. Lab. & Clin. Med.* **3**:40, 1917. Kimball, O. P., and Marine, D.: The Prevention of Simple Goiter in Man (Second paper), *Arch. Int. Med.* **22**:41 (July) 1918. Kimball, O. P., Rogoff, J. M., and Marine, D.: The Prevention of Simple Goiter in Man (Third paper), *J. A. M. A.* **73**:1873 (Dec. 20) 1919.

TABLE 1.—ANALYSIS OF THE RECORDS OF NEW PUPILS

Date Examined	Total Cases Examined	Total New Cases	Normal		Slight Enlargements		Moderate Enlargements		Marked Enlargements		Adenomas	
			Number	Per Cent.	Number	Per Cent.	Number	Per Cent.	Number	Per Cent.	Number	Per Cent.
April 1917.....	3,872	3,872	1,688	43.6	1,931	49.9	246	6.3	7	0.2	39	1.0
November 1917.....	4,415	1,772	831	47.0	820	46.2	121	6.8				
November 1918.....	4,277	1,873	1,037	55.4	779	41.6	53	2.8	4	0.2	6	0.3
October 1919.....	5,520	2,162	1,415	65.4	679	31.4	67	3.1	1	0.05	1	0.05

TABLE 2.—SUMMARY OF AGE INCIDENCE—NEW PUPILS

Date of Examination	Total New Cases	Age									
		10 - 12		12 - 14		14 - 16		16 - 18		18 - 20	
		Number of Cases	Per Cent.	Number of Cases	Per Cent.	Number of Cases	Per Cent.	Number of Cases	Per Cent.	Number of Cases	Per Cent.
April 1917.....	3,872	945	24.4	1,261	32.6	1,140	29.4	453	11.7	73	1.0
November 1918.....	1,873	766	40.9	590	31.5	406	21.7	94	5.0	17	0.9
October 1919.....	2,162	969	44.8	678	31.4	401	18.6	102	4.7	12	0.5

TABLE 3.—RELATION OF AGE TO THYROID CONDITION—NEW PUPILS—1917

	Age									
	10 - 12		12 - 14		14 - 16		16 - 18		18 - 20	
	Number of Cases	Per Cent.	Number of Cases	Per Cent.	Number of Cases	Per Cent.	Number of Cases	Per Cent.	Number of Cases	Per Cent.
Normal.....	530	56.1	521	41.3	460	40.3	156	34.4	21	28.8
Slightly enlarged.	394	41.7	680	53.9	578	50.7	235	51.9	44	60.3
Moderately enlarged.....	21	2.2	59	4.7	98	8.6	60	13.2	8	11.0
Markedly enlarged.....	1	0.1	4	0.3	2	0.4		

TABLE 4.—RELATION OF AGE TO THYROID CONDITION—NEW PUPILS—1918

	Age									
	10 - 12		12 - 14		14 - 16		16 - 18		18 - 20	
	Number of Cases	Per Cent.	Number of Cases	Per Cent.	Number of Cases	Per Cent.	Number of Cases	Per Cent.	Number of Cases	Per Cent.
Normal.....	491	64.2	295	50.0	214	52.7	39	41.5	9	52.9
Slightly enlarged.	267	34.8	276	46.8	168	41.4	49	52.1	6	35.3
Moderately enlarged.....	8	1.0	19	3.2	24	5.9	6	6.4	2	11.8
Markedly enlarged.....	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0

mately 95 per cent. are included in the age group from 10 to 12. Details of the relation of age to the clinical condition of the thyroid are given in Tables 3, 4 and 5.

TABLE 5.—RELATION OF AGE TO THYROID CONDITION—NEW PUPILS—1919

	Age									
	10 - 12		12 - 14		14 - 16		16 - 18		18 - 20	
	Number of Cases	Per Cent.	Number of Cases	Per Cent.	Number of Cases	Per Cent.	Number of Cases	Per Cent.	Number of Cases	Per Cent.
Normal.....	743	76.7	419	61.8	199	49.6	53	51.9	7	58.3
Slightly enlarged.	215	22.2	239	35.3	170	42.4	43	42.2	5	41.7
Moderately enlarged.....	11	1.1	20	2.9	31	7.8	6	5.9	0	0.0
Markedly enlarged.....	0	0.0	0	0.0	1	0.2	0	0.0	0	0.0

TABLE 6.—ANALYSIS OF RECORDS OF NEW PUPILS—NEGROES—1919

	Age					
	10 - 12		12 - 14		14 - 16	
	Number of Cases	Per Cent.	Number of Cases	Per Cent.	Number of Cases	Per Cent.
Normal.....	5	50.0	7	53.9		
Slightly enlarged.....	4	40.0	5	38.5	3	75.0
Moderately enlarged.....	1	10.0	1	7.6	1	25.0

For reference and comparison the original survey of all pupils (April, 1917), is given in Table 3, while in Tables 4 and 5 are given the results of the surveys of new pupils for 1918 and 1919, respectively. It should be emphasized, that in the 1917 examination, 43.9 per cent. of the girls in the 10-12 years age group had enlarged thyroids; that in the 1918 examination 35.8 per cent. had enlarged thyroids; and that in the 1919 examination 23.2 per cent. had thyroid enlargements. This is important from the standpoint of the age at which the prophylactic treatment should be started. When this work was begun, no data of this kind were available, and the fifth grade was arbitrarily chosen as the lower limit, because our limited facilities made it necessary to confine our efforts to what seemed to be the most important age periods. We have seen only forty instances of moderately enlarged glands and no instance of marked enlargement in the 10-12 years age group, and as very striking therapeutic effects are seen in these slight hyperplasias it makes little difference in the ultimate result. If, however, one had to depend entirely on prevention it would be necessary to begin at an earlier age.

There appears to be no noteworthy difference in the incidence of thyroid enlargements between white and colored children. The data are, however, insufficient for any definite conclusion. The data on the twenty-seven colored children are given in Table 6.

EFFECT OF PROPHYLACTIC TREATMENT

The prophylactic treatment as carried out for the past two years consists of the administration of 2 gm. sodium iodid, given in 0.2 gm. doses daily, for ten consecutive school days, repeated each spring and autumn. The general data of those pupils not taking the treatment are given in Table 7, and of those taking the treatment in Table 8. Only pupils with two or more consecutive examinations have been included in the tabulations. A considerable number of pupils, both taking and not taking the treatment, have been omitted because they missed one examination, although otherwise their records were complete. Two thousand, three hundred and five pupils are included in the tabulation

TABLE 7.—RECORD OF PUPILS NOT TAKING PROPHYLACTIC TREATMENT

Time Under Observation, Mos.	Normal				Slightly Enlarged						Moderately Enlarged					
	Unaltered		Increased		Unaltered		Increased		Decreased		Unaltered		Increased		Decreased	
	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
6	47	50.0	47	50.0	93	69.4	36	26.9	5	3.7	16	69.6	7	30.4	0	0.0
12	420	75.5	136	24.5	251	70.3	35	9.8	71	19.9	17	65.4	8	30.8	1	3.8
18	103	65.2	55	34.8	108	74.5	18	12.3	19	13.1	11	57.9	3	15.8	5	26.3
24	135	76.7	41	23.3	106	79.7	8	6.0	19	14.3	9	60.0	3	20.0	3	20.0
30	205	75.1	68	24.9	140	73.7	30	15.8	20	10.5	4	66.7	0	0.0	2	33.3

of those not taking treatment, and 2,190 in the tabulation of those taking treatment. Further, it was necessary to tabulate the results with reference to the length of time under observation. As the prophylactic treatment was given at intervals of six months, we have used this interval as the unit and grouped the pupils according to the periods under observation, 6, 12, 18, 24 and 30 months respectively. Only the results of three groups (normals, slightly enlarged, and moderately enlarged) are included because the fourth group (markedly enlarged) is too small. A comparison of the two tables brings out striking differences between those not taking and those taking iodine. These differences are manifested both in *prevention* of enlargement and in a *decrease* in the size of existing enlargements, i.e., therapeutic effect.

Prevention.—This effect is shown in the columns marked "unchanged" and "increased." Taking the totals for the five six month periods (Table 9) the following results were obtained. Of those that were normal at the first examination and did not take iodine, 347, or 27.6 per cent., have enlarged thyroids, while of those that were normal at the first examination and took iodine as outlined, two, or 0.2 per cent.,

have enlarged thyroids. These two instances of enlargement were investigated. The first pupil, M. T., age 16, had her thyroid examined and classified as normal May 2, 1917, Oct. 17, 1918 and Dec. 3, 1918. At the examination Oct. 15, 1919, it was classified as slightly enlarged. This girl had taken 2 gm of sodium iodid during each of the five possible periods, May, 1917, November, 1917, May, 1918, December, 1918 and May, 1919. A special examination was made Jan. 13, 1920. The enlargement of the thyroid was verified. The enlargement was acquired as opposed to congenital, as shown by the absence of a pyramidal process or thyroglossal tract. The tonsils were markedly enlarged, nearly meeting in the midline when the mouth was widely opened.

TABLE 8.—RECORD OF PUPILS TAKING PROPHYLACTIC TREATMENT

Time Under Observation, Mos.	Normal				Slightly Enlarged						Moderately Enlarged					
	Unaltered		Increased		Unaltered		Increased		Decreased		Unaltered		Increased		Decreased	
	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
6	17	94.4	1	5.6	54	69.2	1	1.3	23	29.5	9	81.8	0	0.0	2	18.2
12	344	99.7	1	0.3	187	45.5	0	0.0	224	54.5	10	23.8	0	0.0	32	76.2
18	73	100.0	0	0.0	72	52.3	1	0.7	64	46.7	7	28.0	0	0.0	18	72.0
24	184	100.0	0	0.0	72	37.9	1	0.5	117	61.6	2	7.7	0	0.0	24	92.3
30	288	100.0	0	0.0	92	28.5	0	0.0	231	71.5	1	2.6	0	0.0	38	97.4

TABLE 9.—SUMMARY—RECORDS OF PUPILS TAKING AND NOT TAKING PROPHYLACTIC TREATMENT

	Taking		Not Taking	
	Totals	Per Cent.	Totals	Per Cent.
Normal:				
Unchanged.....	906	99.8	910	72.4
Increased.....	2	0.2	347	27.6
Slightly Enlarged:				
Unchanged.....	477	41.9	698	72.8
Increased.....	3	0.3	127	13.3
Decreased.....	659	57.8	134	13.9
Moderately Enlarged:				
Unchanged.....	29	20.3	57	64.0
Increased.....	0	0.0	21	23.6
Decreased.....	114	79.7	11	12.4
Total.....	2,190	2,305	

They were abnormally hyperemic, and on direct questioning the pupil stated she was subject to recurrent tonsillitis. There was also slight enlargement of the lymphoid tissue at the base of the tongue and in the nasopharynx. The general impression was that of a neurotic individual with general lymphoid hyperplasia.

The second girl, aged 15, had her thyroid first examined and classified as normal Nov. 27, 1918. At the examination Oct. 16, 1919, it was classified as slightly enlarged. This girl had taken 2 gm. sodium iodid during each of the two available periods, November, 1918 and May, 1919. A special examination was made Jan. 13, 1920, and the thyroid

enlargement was verified. Careful inspection revealed the presence of Hutchinson teeth, depressed nasal arch and interstitial keratitis. We considered the case one of neglected congenital syphilis.

Passing to Group 2, or those classified as having slightly enlarged thyroids at the first examination, it is seen among those not taking the prescribed treatment that 127, or 13.3 per cent., underwent further enlargement, while of those taking the prescribed treatment, three, or 0.3 per cent., underwent further enlargement. Two of these three pupils were again examined Jan. 13, 1920. One, R. R., aged 14, was examined May 2, 1917, Oct. 12, 1917 and Nov. 26, 1918, and the thyroid classified as slightly enlarged, and at the examination Oct. 16, 1919 the gland was classified as moderately enlarged. This girl had taken the prescribed treatment only during the last three available periods, May, 1918, November, 1918 and May, 1919. A special examination was made Jan. 13, 1920 and the thyroid enlargement verified. In this case also the tonsils were enlarged and the seat of recurrent infections. The second case, V. S., aged 11, was examined Oct. 22, 1917 and Nov. 27, 1918 and the thyroid classified as slightly enlarged. At the third examination, Oct. 16, 1919, it was classified as moderately enlarged and the special examination Jan. 13, 1920, verified this finding. This girl had taken the prescribed treatment during the four available periods, November, 1917, May, 1918, December, 1918, and May, 1919. Superficial inspection failed to reveal the existence of any associated pathologic condition as was found in each of the first three cases mentioned. The fifth girl was not present for the special examination. These five cases are the only instances out of 2,190 pupils taking iodine that showed enlargement. For the group with slightly enlarged thyroids taking iodine, 447, or 41.9 per cent., remained unchanged, while of those not taking iodine, 698, or 72.8 per cent., remained unchanged.

Passing to the third group, or those classified as having moderately enlarged thyroids at the first examination, it is seen that of those taking iodine, twenty-nine, or 20.3 per cent., remained unchanged, while of those not taking iodine, fifty-seven, or 64.0 per cent., remained unchanged; of those taking iodine none increased, while of those not taking it, twenty-one, or 23.6 per cent., increased.

Curative or Therapeutic Effect.—Although of secondary importance, the results are just as striking as those above described under prevention. These results are shown in the column marked "decreased." Of those pupils whose thyroids were classified as slightly enlarged at the first examination, and who took iodine, 659, or 57.8 per cent., definitely decreased in size, while of those not taking the prescribed treatment, 134, or 13.9 per cent., decreased. Passing to the group

whose thyroids were classified as moderately enlarged at the first examination, 114, or 79.7 per cent., of those taking iodine showed definite decreases. In some the decrease in size was most striking and hardly to be believed had we not had actual measurements and descriptions of the condition previously. The reduction in several cases was as marked as one sees in the thyroid enlargement of young dogs, sheep or cattle following the use of iodine. It means that with similar anatomic conditions, i.e., uncomplicated hyperplasias of the thyroids, the degree of reaction is similar. Ordinarily, one does not obtain the striking therapeutic effect on human thyroid enlargements that is seen in animals. This, as pointed out in previous papers, is due, in large part, to the duration of the enlargement, the presence of adenomas, cysts, degenerations, hemorrhage, etc., which are common in all long standing human goiters, while very uncommon in the lower animals at the ages when these animals are usually observed. The therapeutic effect is a very

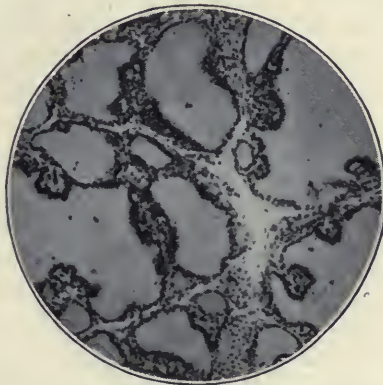


Fig. 1.—Moderate active hyperplasia; control specimen removed ten days after beginning administration of iodine (D—185a).

important supplement to prevention and makes it possible to begin prophylactic treatment in older pupils with the same practical result than would otherwise be possible.

DISCUSSION

Our observations on the prevention of simple goiter in man have extended over a period of thirty months. The disease is as easily prevented in man as in fish or in domestic animals.

Of 2,190 pupils taking 2 gm. sodium iodide twice yearly, five have shown enlargement of the thyroid, while of 2,305 pupils not taking the prophylactic, 495 have shown enlargement of the thyroid. Of 1,182 pupils with thyroid enlargement at the first examination and who took the prophylactic, 773 thyroids have decreased in size, while of 1,048 pupils with thyroid enlargement at the first examination and who did not

take the prophylactic, 145 thyroids have decreased in size. These figures demonstrate in a striking manner both the preventive and the therapeutic effects. There is an error in the above figures in that many pupils listed as not taking iodine have taken iodine in one or another form outside the school jurisdiction. No attempt has been made to detect or estimate this error.

In the practical application of the preventive treatment, one must keep in mind the three periods when simple thyroid enlargements most commonly occur, viz., (1) fetal, (2) adolescence and (3) pregnancy.

(1) Prevention of goiter in mother and fetus is as simple as that occurring during adolescence. Practically, it would seem that it is a charge or responsibility of individual members of the medical profession supplemented with public education.

(2) The prevention of goiter of adolescence, on the other hand, should be a public health measure under state, county or municipal

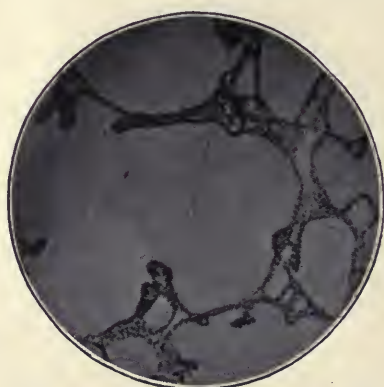


Fig. 2.—Same gland as Figure 1; specimen removed forty-four days after beginning administration of iodine.

control. The existing systems of organization of the schools, public and private, is sufficient to handle all the details without additional aid or expense. Education of the pupils would be combined with the actual administration so that after leaving school they could continue the treatment, if necessary. Physicians in industrial medicine could render an important service in this field. Thyroid enlargement is approximately six times as frequent in girls as in boys. It is a social economic question each community must decide whether it will include both sexes. Likewise, as to the age of beginning and stopping the use of iodine. In this climate probably the maximum of prevention, coupled with the minimum of effort, would be obtained by giving it between the ages of 11 and 17 years. As applied to our schools it would mean beginning with the fifth grade.

Manner and Form of Administration.—As previously stated, iodine is taken up by the thyroid gland when given by mouth, by inhalation, or by external application. Weith² reports favorable therapeutic effects from inhalation of iodine as carried out by suspending a wide mouthed bottle containing a 10 per cent. tincture of iodine in the school room. Waste and lack of control of amounts taken are the most obvious objections. Similar objections hold in case of external application. Some form of oral administration seems most practical and economical. The addition of iodine or a salt of iodine to the water supply as we have done in preventing goiter in fish might be considered. There are obvious objections to such a plan. It would entail enormous waste. It is applicable only when there are installations, i. e., in towns and cities, and depending on the chemical impurities in water interactions might throw out the iodine. The most feasible oral method would seem to be the individual administration of definite small amounts, either in solution or as tablets. The cheapest salt, sodium iodide, could be given in either form. Manufacturing pharmacists state that sodium iodide could be prepared very cheaply in tablet form protected from the action of water and light. For private use, the well known U.S.P. preparations, syrup of ferrous iodide and syrup of hydriodic acid are excellent.

Amounts of Iodine to be Used.—An ounce of syrup of ferrous iodide or hydriodic acid given over a period of from two to three weeks and repeated twice yearly would seem ample. As a public health measure, we have used 2 gm. of sodium iodide given over a period of two weeks and repeated twice yearly. This dosage has prevented enlargement of the thyroid in more than 99 per cent. of the children in this mildly goiterous district. It is our opinion that much smaller amounts would suffice for healthy children and healthy pregnant women, provided the period of taking was prolonged, i. e., 1 gm. sodium iodide distributed over a month would accomplish as good thyroid effects as 2 gm. given over a period of two weeks.

The prevention of thyroid enlargement in individuals with other diseases or residing in extremely goiterous districts, as in some glacial valleys of Alaska and British Columbia; certain districts in the Alps and Himalayas, might require larger amounts of iodine for normals than above indicated. Our data of the clinical condition of four of the five cases that enlarged during the administration of 2 gm. of sodium iodide, twice yearly, suggest that in infections (chronic catarrhal or suppurative tuberculosis, syphilis, etc.) and possibly also in conditions like chlorosis, osteomalacia, lymphatism and exophthalmic goiter, such amounts might not control the thyroid growth. In such conditions there

2. Weith: Goiter and Iodine in the School, Cor.-Bl. f. schweiz. Aerzte 49: 1474, 1919.

may be a greatly increased demand for the thyroid hormone or the organism's ability to store iodine in the thyroid may be impaired. There is a great deal of clinical evidence for the first view and none at present in support of the second.

Effect of Iodine on the Thyroid Gland.—This is manifested in two ways (1) on the iodine store and (2) on the histologic condition. Both of these effects have been fully described in previous papers.³

Effect on the Store: If the thyroid gland is not saturated with iodine (i. e., contains less than 4 mg. per gm. of dried gland) it is taken up readily by the cells following its administration in any form and in any manner thus far studied. An increase in the iodine content of thyroid may be demonstrated in a few seconds following the injection of a soluble salt into the circulation.⁴ Iodine thus taken up is held by the cells until elaborated into the physiologically active hormone, when any

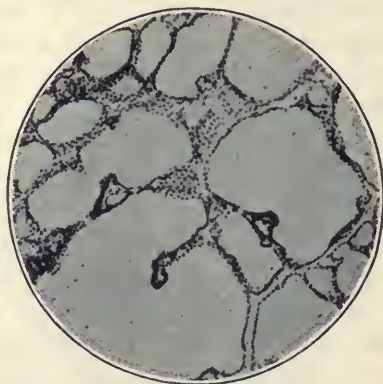


Fig. 3.—Same gland as Figures 1 and 2; specimen removed seventy-eight days after beginning administration of iodine. Total amount of iodine given, 90 mg.

excess is excreted into the follicular spaces and stored in the so-called colloid. Two factors then are concerned in the storage of iodine in the thyroid: (a) the capacity of the gland cells to take up and elaborate the

3. Marine, D.: On the Occurrence and Physiological Nature of Glandular Hyperplasia of the Thyroid (Dog and Sheep) Together with Remarks on Important Clinical Human Problems, *Bull. Johns Hopkins Hosp.* **18**:359, 1907. Marine, D., and Lenhart, C. H.: Colloid Glands (Goiters): Their Etiology and Physiological Significance, *Bull. Johns Hopkins Hosp.* **20**:131, 1909. Marine, D., and Lenhart, C. H.: Effects of the Administration or the Withholding of Iodine Containing Compounds in Normal, Colloid or Actively Hyperplastic Thyroids of Dogs, *Arch. Int. Med.* **4**:253, 1909. Marine, D.: Quantitative Studies on the in vivo Absorption of Iodine by Dogs' Thyroid Glands, *J. Biol. Chem.* **22**:547, 1915.

4. Marine, D., and Rogoff, J. M.: The Absorption of Potassium Iodide by the Thyroid Gland in vivo Following Its Intravenous Injections in Constant Amounts, *J. Pharm. & Exper. Therap.* **8**:439, 1916.

hormone and (b) the capacity of the colloid material to store the product. It is evident, then, that to obtain maximum thyroid effects from a minimum amount of iodine, it should be administered in amounts not to exceed the capacity of the cells at any given time to handle it. As has been shown, the elaboration of the hormone proceeds slowly⁵ in the most active thyroids. Also when one recalls that from 4 to 5 mg. of iodine per gm. of dried gland, or from 25 to 30 mg., is the total storage capacity of a normal thyroid, it is clear that small amounts of iodine (a few mg.) given daily for a long period of time (a month or more) would produce optimum thyroid effects. In the school work, a compromise was found necessary, increased amounts and decreased time of administration.

Effect on Histology of the Thyroid: It has been shown that the minimum amount of iodine store necessary to maintain normal or quiescent thyroid structure is quite constant for mammals.⁶ In the dog, sheep, human and pig thyroid it is approximately 1 mg. per gm. of dried gland, and immediately the percentage is reduced below the minimum, hypertrophic and hyperplastic changes begin and continue until the store of iodine has again been raised above the minimum requirements, when involution takes place. This cycle may be repeated many times in the same individual under natural or experimentally controlled conditions. In young dogs, with active hyperplasia, involution is usually complete in from fourteen to twenty-one days after beginning the administration of iodine. The histologic features of this involution have been described in detail in other papers, but for reference three microphotographs illustrating it are reproduced (Figs. 1, 2 and 3).

Untoward Effects.—No obvious case of exophthalmic goiter has developed, although such cases have been carefully looked for. An occasional instance of iodine idiosyncrasy (iodism), amounting to less than 0.5 per cent. of the cases, was noted. Most of the cases were very mild and the girls did not stop the treatment. As an untoward effect it is negligible.

SUMMARY.

Observations on the prevention of simple goiter in man on a large scale have extended over a period of thirty months. The results show that it may be prevented very simply and cheaply in normal individuals. While thyroid enlargements of adolescence are more common, they are

5. Marine, D., and Rogoff, J. M.: How Rapidly Does the Intact Thyroid Gland Elaborate Its Specific Iodine Containing Hormone? *J. Pharm. & Exper. Therap.* **9**:1, 1916.

6. Marine, D., and Williams, W. W.: Relation of Iodine to the Structure of the Thyroid Gland, *Arch. Int. Med.* **1**:349, 1908. Marine, D., and Lenhart, C. H.: Further Observations on the Relation of Iodine to the Structure of the Thyroid Gland in the Sheep, Dog, Hog and Ox, *Arch. Int. Med.* **3**:66, 1909

not more important than those occurring in mother and fetus. Prevention of adolescent goiter is properly a public health problem, while the prevention of fetal and maternal thyroid enlargements is largely a responsibility of individual physicians. The presence of pathologic conditions may modify the result of the prophylactic treatment in individual cases. While such instances are rare they are important and merit detailed reports.

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Goiter Survey Work in Ohio: The Incidence of Simple Goiter in the School Children of Cleveland, Akron and Warren*

David Marine, M. D., New York City, and O. P. Kimball, M. D., Warren.

Editor's Note.—In the July issue of *The Journal* the survey work of Drs. McCord and Walker of Cincinnati on the incidence of endemic goiter and its prevention in school children was published with a view to interesting the profession at large in this public health activity. The present survey of goiter incidence in the school children of Cleveland, Akron and Warren, is herewith added to the papers of the Goiter Symposium to show just how important and necessary the work of goiter prevention is. Ultimately it is expected that further surveys will enable the Public Health Service to handle the problem involved effectively. A comparison of the incidence of thyroid enlargements in relation to sex shows that in the groups of school children examined by Drs. Marine and Kimball it is a little more than twice as frequent in girls. The investigators conclude, however, that the general statement that sex makes no difference in the incidence of thyroid enlargements before puberty and that during and after puberty it becomes five or six times more frequent in girls does not conflict with their figures. The explanation for the apparent variance is that the majority of the pupils examined were below the age of puberty.

THE Great Lakes basin, including the St. Lawrence river valley, is the most important district of endemic or simple goiter in North America. Barton¹ in 1800, wrote an excellent monograph on the occurrence of goiter among the American Indians living along the southern shore of Lakes Ontario and Erie. Osler² has emphasized its frequency in the Province of Ontario; Adami³ in the St. Lawrence valley; Dock⁴ in Michigan, and many other observers including ourselves have published papers on endemic goiter in man and animals living in the states bordering on the Great Lakes.

As compared with the severe endemic goiter regions of the world, *e. g.*, the Alpine districts of France, Italy, Switzerland and Austria or the Himalaya region of northern India, the Great Lakes basin would be classed as a mild endemic goiter district. This is shown by the great rarity of cretinism, which, as Morel⁵ first emphasized, is the end stage of severe goiter, (*i. e.*, thyroid insufficiency).

OBJECTS OF THE PRESENT SURVEY

While the frequency of simple goiter in man in

*From the Department of Experimental Medicine, Western Reserve University. Aided by a grant from the Committee on Therapeutic Research of the Council on Pharmacy and Chemistry of the American Medical Association..

northern Ohio is generally known, its actual incidence in any locality or unit of population is entirely unknown, except for the papers by ourselves on the incidence in school girls of Akron. Our object in publishing the following data, therefore, is first to encourage the extension of this survey work so that ultimately a map showing the actual incidence may be prepared as part of the *Public Health Record* and secondly, since simple goiter is so easily prevented, such a survey would be necessary in order to determine where goiter prevention as a public health measure is most needed.

DATA OF THE SURVEY

The data of the survey are given in the following tables. In Cleveland only the pupils of three schools from different sections of the city were examined. In Warren and Akron, the pupils of all schools were examined. The examinations in Warren and Cleveland include boys and girls, while in Akron only girls were examined. No pupils below the fifth grade were examined. Fifth grade pupils average 10 to 11 years of age and the great increase in the incidence of thyroid enlargement in this climate begins about the age of 13 to 14 years or in the seventh to eighth grades.

TABLE I.
Summary of Age Incidence of Pupils.

Schools	Total Cases	10—12 Years		12—14 Years		14—16 Years		16—18 Years		18—20 Years	
		No.	Per cent	No.	Per cent	No.	Per cent	No.	Per cent	No.	Per cent
						GIRLS.					
Cleveland	406	164	40.39	172	42.37	65	16.01	5	1.23	0	0.00
Iron	7907	2680	33.89	2529	31.99	1947	24.62	649	8.21	102	1.29
Warren	925	210	22.70	267	28.87	275	29.73	153	16.54	20	2.16
						BOYS.					
Cleveland	273	84	30.77	132	48.35	56	20.51	1	0.37	0	0.00
Warren	911	191	20.97	314	34.47	250	27.44	131	14.38	25	2.74

TABLE II.

Relation of Age to Number of Pupils with Normal Thyroids.

Schools	Total Cases	10—12 Years		12—14 Years		14—16 Years		16—18 Years		18—20 Years	
		No.	Per cent	No.	Per cent	No.	Per cent	No.	Per cent	No.	Per cent
						GIRLS.					
Cleveland	254	117	46.06	102	40.16	34	13.39	1	0.39	0	0.00
Akron	4157	1764	42.43	1235	29.71	873	21.00	248	5.97	37	0.89
Warren	699	187	26.75	208	29.76	203	29.04	89	12.73	12	1.72
						BOYS.					
Cleveland	223	69	30.94	112	50.22	41	18.39	1	0.45	0	0.00
Warren	824	176	21.36	283	34.35	222	26.94	119	14.44	24	2.91

TABLE III.

Relation of Age to Number of Pupils with Slightly Enlarged Thyroids.

Schools	Total Cases	10—12 Years		12—14 Years		14—16 Years		16—18 Years		18—20 Years	
		No.	Per cent	No.	Per cent	No.	Per cent	No.	Per cent	No.	Per cent
						GIRLS.					
Cleveland	132	46	34.85	57	43.18	25	18.94	4	3.03	0	0.00
Akron	3269	876	26.80	1195	36.56	816	24.96	327	10.00	55	1.68
Warren	199	19	9.55	55	26.74	63	31.66	54	27.13	8	4.02
						BOYS.					
Cleveland	42	8	19.05	20	47.62	14	33.33	0	0.00	0	0.00
Warren	84	15	17.86	30	35.71	27	32.14	11	13.10	1	1.19

TABLE IV.

Relation of Age to Number of Pupils with Moderately Enlarged Thyroids.

Schools	Total Cases	10—12 Years		12—14 Years		14—16 Years		16—18 Years		18—20 Years	
		No.	Per cent	No.	Per cent	No.	Per cent	No.	Per cent	No.	Per cent
						GIRLS.					
Cleveland	20	1	5.00	13	65.00	6	30.00	0	0.00	0	0.00
Akron	373	40	10.73	98	26.27	153	41.02	72	19.30	10	2.68
Warren	26	4	15.38	4	15.38	9	34.62	9	34.62	0	0.00
						BOYS.					
Cleveland	8	7	87.50	0	0.00	1	12.50	0	0.00	0	0.00
Warren	3	0	0.00	1	33.34	1	33.33	1	33.33	0	0.00

TABLE I, shows the number of pupils examined and the groups according to age and sex. Each of the five age groups includes two years. This gives the range of the ages included in the particular fraction of the population of each community dealt with.

The distribution of pupils with *normal* thyroids, with *slightly enlarged* thyroids, with *mod-*

erately enlarged thyroids, and with *markedly enlarged* thyroids in relation to age is shown in TABLES II, III, IV, and V. The all important factor in such grouping is the definition or standard of the normal thyroid clinically. The other groups—slightly enlarged, moderately enlarged and markedly enlarged are purely arbitrary and relative divisions. Such a grouping is exposed

Schools	Total Cases	10—12 Years		12—14 Years		14—16 Years		16—18 Years		18—20 Years	
		No.	Per cent	No.	Per cent	No.	Per cent	No.	Per cent	No.	Per cent
						GIRLS.					
Cleveland	0	0	0.00	0	0.00	0	0.00	0	00.0	0.	0.00
Akron	8	0	0.00	1	12.50	5	62.50	2	25.00	0	0.00
Warren	1	0	0.00	0	0.00	0	0.00	1	100.00	0	0.00

TABLE VI.
Summary of Goiter Survey Records.

Schools	Total Cases Examined	Normal		Slightly Enlarged		Moderately Enlarged		Markedly Enlarged		Adenomas	
		No.	Per cent	No.	Per cent	No.	Per cent	No.	Per cent	No.	Per cent
						GIRLS.					
Cleveland	406	253	62.31	133	32.76	20	4.93	0	0.00	7	1.72
Akron	9679	4971	51.36	4209	43.49	487	5.03	12	0.12	46.	0.48
Warren	925	699	75.57	199	21.51	26	2.81	1	0.11	4	0.43
						BOYS.					
Cleveland	273	223	81.68	49	17.95	1	0.37	0	0.00	0	0.00
Warren	911	824	90.45	84	9.22	3	0.33	0	0.00	1	0.11

to the error of the personal bias of the observer and this cannot be eliminated even with the elaborate systems of thyroid measurements suggested from time to time. The plan we have used is relative and if the standard of normal remains constant and the same observer makes all examinations the relative importance of the data is not impaired.

STANDARDS OF NORMAL AND ENLARGED THYROIDS

Our standard for the normal adult thyroid is that the weight does not exceed 25 grams. Most text books of anatomy and many individual observers quote higher weights for normal. Clinically no part of the outline of a normal thyroid can be detected on inspection. The lateral lobes cannot be palpated. The normal isthmus can be felt as a very thin band across the trachea in all individuals except the very stout and those in whom it lies on a level with or behind the upper border of the manubrium. In such cases it may be felt by having the pupil swallow while the observer holds the thumb against the trachea. This portion of the gland is very superficial and slight enlargements are often visible as a transverse ridge before the enlargement of the lateral lobes is palpable or visible. As the thyroid usually undergoes uniform enlargement the condition of the isthmal or palpable portion is a safe index and standard for the detection of the slight enlargements which because they are so common

are usually not considered as enlarged by local physicians.

TABLE VI, is a summary giving the total *normal*, *slightly enlarged*, *moderately enlarged*, and *markedly enlarged* thyroids for the three communities. Adenomas are also included. The percentages shown in this table for Cleveland are misleading because the number and age range of pupils examined is inadequate. No pupils above the 8th grade were examined in the three Cleveland schools, while in Akron and Warren all pupils from the 5th to the 12th grades were examined. The number and age range for both Akron and Warren are adequate and give a fair picture of the percentage relations of normals, slightly enlarged, moderately enlarged and markedly enlarged thyroids.

SUMMARY

In the three communities of Akron, Cleveland, and Warren, Ohio, the percentage relations of school children with normal and enlarged thyroids were found to be as follows: (1) *Girls*: 9679 (examinations extending through 3 years) were examined in Akron—51.36 per cent. had normal thyroids and 48.64 per cent had enlarged thyroids; 406 were examined in Cleveland—62.31 per cent. had normal thyroids and 37.69 per cent. had enlarged thyroids; 925 were examined in Warren—75.57 per cent. had normal thyroids and 24.43 per cent had enlarged thyroids. (2)

Boys: 273 were examined in Cleveland—81.68 per cent had normal thyroids and 18.32 per cent had enlarged thyroids; 911 were examined in Warren—90.45 per cent. had normal thyroids 9.55 per cent. had enlarged thyroids. *A comparison of the incidence of thyroid enlargement in relation to sex shows that in the groups of school children we have examined it is a little more than twice as frequent in girls.* The general statements that sex makes no difference in the incidence of thyroid enlargements before puberty and that during and after puberty it becomes five or six times more frequent in girls do not

conflict with our figures. The explanation for the apparent variance is that the majority of the pupils examined were below the age of puberty.

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MONTEFIORE HOME AND HOSPITAL.

THE INFLUENCE ON THE THYROID OF ANASTOMOSIS OF THE PHRENIC AND CERVICAL SYMPATHETIC NERVES

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These experiments represent an attempt to repeat the observations of Cannon, Binger and Fitz (1) on the effects of fusion of the anterior root of the phrenic nerve with the cervical sympathetic upon the thyroid function in cats. They state that symptoms resembling those of Graves' disease in man developed. There was marked tachycardia, loose movements of the bowels and falling of the hair. The animals were unusually excitable. The basal metabolism was greatly increased. The pupil was larger on the operated side. In one of the animals exophthalmos and respiratory hippus developed on that side. In one animal in which the symptoms were well developed (2), removal of the thyroid gland on the operated side stopped the progress of the disease. Whereas other animals had died within three months of the first appearance of the symptoms, this cat lived for seven months after the operation, when it was purposely killed. The conclusion is drawn that the symptoms were due to increased thyroid secretion, owing to the bombardment of the gland by impulses discharged along the phrenic synchronously with the respiratory discharge.

Troell (3), working mainly with cats and dogs, stated that he did not find any change in the pupil corresponding to the respiration. But he mentions that in the animals in which he looked for this, the pupil was still contracted and the nictitating membrane still forward on the operated side, indicating that the innervation had not yet been restored at the time the animals were killed. Accordingly it could not be expected that evidence of rhythmical discharges along the phrenic should be obtained on these animals.

Quite recently Burget (4) has reexamined the question, with a negative result. The animals (cats and rabbits) after recovery from the operation were apparently normal in every respect.

Our experiments were made on ten cats. Early in 1916, the central end of the anterior root of the phrenic was sutured by a single stitch end to end to the cephalic end of the cervical sympathetic. One of the animals died one week after the operation from pneumonia; another one month after the operation from the common epidemic disease, characterized by "snuffles," cough, sneezing, poor appetite, progressive emaciation and falling hair, which was prevalent at the time among our stock cats. Two of the cats were killed eight months after the operation on account of the same disease. One was lost after five months. The remaining five cats were in excellent health at the time they were killed (eight, eight and one-half, nine, eleven and one-half and twenty-one and one-half months, respectively, after the operation). Before the animals were sacrificed, the condition of the nerves above and below the site of the anastomosis was carefully tested by electrical stimulation. In all the ten animals systematic and frequent observations were made from the time of operation. In the eight which were kept for several months, the pupil on the operated side had regained equality with its fellow and the nictitating membrane was retracted to the same extent. One of the protocols is reproduced as a sample in condensed form.

Condensed protocol, cat 5, adult, female

January 29, 1916. Phrenic-sympathetic anastomosis made on left side. Between January 30, 1916 and November 17, 1917, when the animal was sacrificed, observations were made frequently during the first year and at less frequent intervals during the rest of the period. Within the first two or three months the contracted pupil on the operated side gradually became more like the pupil on the right (unoperated) side, equality being finally established and maintained until the animal was killed. Reactions to light and accommodation were equal in both eyes. At no time was respiratory hippus observed. Both eyes responded simultaneously and to the same extent to such stimuli as psychical disturbances. No evidence of any abnormal condition was at any time present. The cat became somewhat of a laboratory pet, feeding well and maintaining an excellent nutritional state. On March 12, 1916, she gave birth to five normal kittens. They were reared in and remained a long time about the laboratory. Towards the end of June, 1916, the animal again became pregnant; normal parturition.

November 17, 1917. Pupils equal, react equally to light, no change with respiration. Changes in the size of the left pupil are always accompanied by similar changes in the right. Anesthetized with ether—the pupils remain equal; the left nictitating appears slightly more forward than the right. The pupils react equally to light, as before the anesthetic. No respiratory hippus. Dissected down to the site of the anastomosis; found neuroma uniting the anterior root of

the phrenic with the cephalic end of the cervical sympathetic. The identity of the nerves was verified at autopsy. The various nerves above and below the neuroma were now stimulated repeatedly with a weak interrupted current, which could be just distinctly felt by the tongue. In all, about twenty stimulations were made. Stimulation of the sympathetic cephalad to the neuroma gave marked dilatation of the pupil and retraction of the nictitating. The same result was obtained on stimulating the anterior phrenic root central to the neuroma. In the meantime the phrenic root was not cut. Stimulation of the sympathetic caudad to the neuroma gave no effect on the eye unless the electrodes were very near the neuroma, when a slight dilatation of the pupil, but no retraction of the nictitating, was elicited. Stimulation of the neuroma itself always gave good dilatation of the pupil and retraction of the nictitating. These results were verified several times. Then the anterior root of the phrenic was ligated as high up as possible and cut central to the ligature. It was now again stimulated with the same result as before, namely, good pupil and nictitating reactions. Stimulation of the sympathetic caudad to the neuroma and cephalad to it, respectively, and stimulation of the neuroma gave the same results as before. On ligation and section of the anterior phrenic root it was observed that the left pupil became smaller than the right and remained smaller for the rest of the experiment. The thyroids were removed for histological examination; they were approximately equal in size and color, and appeared normal. The adrenals were normal in appearance and size; the left weighed, 0.212 gram, the right, 0.219 gram. Sections from the two thyroids, hardened and stained in the same manner, showed an identical histological picture, that of normal thyroid.

RÉSUMÉ OF RESULTS

In none of the animals were any symptoms resembling those of Graves' disease observed. In several it was proved by electrical stimulation that functional union had occurred between the phrenic and the cells of the superior cervical ganglion innervating the iris and the nictitating membrane. It was shown in several of the cats that a tonic dilator effect must have been exerted through the phrenic on the pupil of the operated side. This follows from the fact that in animals in which it was demonstrated by electrical stimulation that the phrenic caused dilatation of the pupil, while the sympathetic below the neuroma did not, the pupils on the two sides were equal. In the cat which was allowed to live longest, it was proved directly by nerve section that the phrenic was exerting a tonic dilator effect, for when the anterior phrenic root central to the neuroma was divided the pupil on that side became smaller than the other and remained smaller. In several of the cats the effect of stimulation of sensory nerves (central end of cut sciatic) on the pupil was noted. Dilatation occurred on the operated side at the same time as on the normal side and in the same

degree. In none of the animals was respiratory hippus seen although carefully and repeatedly looked for. If the dilator innervation is due to impulses from the respiratory center, there does not seem to be any obvious reason why a rhythmical change synchronous with the respiration should not be present. We can only state that it was not seen in any of our cats. Exophthalmos did not develop in any of the animals. The thyroids were always approximately equal on the two sides and of the same color on gross examination. Histologically no difference was observed between the thyroid on the operated and that on the normal side. Variations in the weight of the adrenals were within the normal range.

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THE LIBERATION OF THE INTERNAL SECRETION OF THE THYROID GLAND INTO THE BLOOD

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The object of this investigation was to determine whether it is possible to detect, in the blood coming from the thyroid gland, a substance whose physiological activity corresponds to that of the gland. There is at present no evidence which clearly shows the path by which the internal secretion of the thyroid gland enters the blood. Histological investigations of earlier workers led them to believe that the internal secretion reaches the blood through the thyroid lymphatics rather than directly through the blood capillaries, but this view has not been corroborated by more direct evidence.

Carlson and Woelfel (1) have attempted to detect the active substance of the thyroid gland in the lymph coming from the gland, but they did not succeed in obtaining good evidence of the presence of an active compound in the lymph which they collected. Owing to the very slow flow of lymph in the normal thyroid (about 2 to 5 cc. in twenty-four hours, in dogs) they were compelled to employ animals with goiterous glands in which the lymph flow from the thyroid is comparatively large. The lymph was collected through a cannula in the main neck lymph trunk below the point of entrance of the thyroid branches, after tying all of the branches above the thyroid. Chemical tests for iodine made with the thyroid lymphs of ten goiter dogs yielded negative results. Injection of the lymph into normal dogs yielded no definite evidence of the presence of active thyroid substance by the blood pressure or other reactions. Complete elimination of the thyroid and parathyroid lymph for thirty-six

to forty-eight hours in normal foxes did not induce symptoms of thyroidparathyroidectomy.

They also attempted to detect possible thyroid activity in the thyroid lymph by the acetonitrile test of Hunt (2, 3) but the results were again negative. As none of the reactions employed by these investigators was sufficiently sensitive or specific for thyroid, definite results could not have been obtained by them. It must be remembered, also, that the thyroid lymph was obtained from animals with goiters, and if the internal secretion of the thyroid enters the lymph, it is highly probable that lymph from a goiterous gland would differ materially from the lymph of a normal gland.

The results obtained in a number of investigations conducted in this laboratory with the "tadpole reaction" have indicated that this may be a sufficiently sensitive means of detecting such quantities of the internal secretion of the thyroid gland as might leave the gland in the blood. It has been shown by Gudernatsch (4) that when tadpoles are fed with thyroid gland they undergo marked changes manifested by rapid emaciation and acceleration of metamorphosis. Lenhart (5) found that this effect is proportional to the quantity fed and the amount of iodine present in the thyroid and that inorganic iodine does not produce the same effect. We have shown, further (6), that this effect is caused only by the iodine of the thyroid which is in specific combination.

When desiccated normal thyroid gland, containing a good store of colloid and iodine, is fed to tadpoles the emaciation and acceleration of metamorphosis occur so rapidly that it is impossible to observe which phenomenon precedes the other. If the dose is small or the iodine content of the gland is below that usually found in a normal gland, the first indication of activity is generally emaciation or retardation of growth and this is soon followed by angulation of the head. If the thyroid is very low in iodine, only the emaciation or retardation of growth may be observed for some time and finally the change in shape of the body and the appearance of the legs occurs somewhat sooner than in the control tadpoles. In our previous work it has been fre-

quently observed that when a specimen of thyroid that is very low in iodine is used or when a very minute dose of a more active thyroid is fed to tadpoles the principal indication of activity is emaciation or retardation of growth, the other changes occurring at about the same time as in the control tadpoles. It has been observed by Barfurth (7) that overfeeding with indifferent food causes metamorphosis to be postponed. This fact must not be overlooked, for when a preparation of thyroid with a very low iodine content is used a relatively large amount is generally offered to the tadpoles, and since the excessive amount of inert food is capable of postponing metamorphosis, the emaciation or retardation of growth is the only evidence of activity that might occur. Indeed, as already indicated, this is in accord with our experience. The reaction of tadpoles to the specific iodine compound of the thyroid is much more sensitive than the chemical tests at present available. In addition to the possibility of detecting the presence of small amounts of thyroid substance the quantitative character of the reaction renders it especially valuable as a means for determining the pharmacological activity of thyroid, by biological assay (8).

In this paper are reported the results of a preliminary set of experiments with blood obtained from the thyroid glands of three dogs, in which the tadpole reaction was employed. All of the blood specimens, after clotting, were dried at 55°C., and ground into a fine powder. The thyroid lobes of these animals were also dried and powdered, a small piece of each lobe having first been preserved for histological examination. A description of the procedures employed in obtaining the thyroid bloods is given in the following protocols.

Protocol—Dog 1

December 20, 1917. Adult male, weight 12.1 kgm.; thyroid lobes large and very vascular. Anesthetised with ether and dissected down, exposing both thyroid lobes. Isolated the right vago-sympathetic nerve in the neck. Inserted an oiled cannula into the vein at the upper pole of the right thyroid lobe and, without stimulation of the vago-sympathetic, collected thyroid blood (A); 136.5 grams of blood

was obtained in six and one-half minutes (21 grams per minute). As the blood in this cannula was clotting, another cannula was inserted into the vein at the lower pole of the same gland and the upper pole tied off. The right vago-sympathetic nerve was now ligated and cut and thyroid blood (B) was collected for two and one-half minutes during stimulation of the cephalic end of the vago-sympathetic nerve and for one-half minute after the stimulation was discontinued; 200 grams of blood was obtained in three minutes (66.7 grams per minute). The characteristic pallor of the gland due to vaso-constriction was not observed during the stimulation. The blood flow became greater after the upper pole was tied off. Now collected (through the same cannula) for five minutes without stimulation, specimen (C); 129.5 grams of blood was obtained (25.9 grams per minute). Clotting in the cannula occurred during the collection of this specimen. Finally obtained blood from the left iliac vein (D), and another specimen from the right thyroid artery (E).

The right thyroid lobe weighed 14.8 grams, the left lobe weighed 14.6 grams. A specimen of each lobe was saved for histological examination and the rest was desiccated and powdered, and preserved for feeding experiments and iodine determinations. No detectable iodine was found in these glands.

The blood specimens were allowed to clot and were placed with the thyroid lobes into the drying oven at 55°C. When completely dried they were powdered and saved for iodine determinations and feeding experiments. No detectable iodine was found in the amounts of blood available for testing.

On histological examination the thyroid lobes showed marked hyperplasia.

Protocol—Dog 2

January 8, 1918. Adult male; weight 10.87 kgm. Thyroid lobes small. Under ether anesthesia exposed both thyroid lobes; isolated the left vago-sympathetic nerve. Inserted an oiled cannula into the vein at the upper pole of the left thyroid lobe, clipped off the vein at the lower pole and collected through the cannula a specimen of thyroid blood (A): 152.0 grams of blood was obtained in three and one-half minutes (43.4 grams per minute). Now ligated and cut the left vago-sympathetic nerve and collected (through the same cannula) another specimen of thyroid blood (B): 69.5 grams of blood was obtained in 5 minutes (14.0 grams per minute). A clot formed in the cannula during the collection of the blood. The vein was tied off close to the cannula and an-

other cannula inserted into the same vein near the gland. During off and on stimulation of the cephalic end of the vago-sympathetic nerve collected specimen (C). The characteristic slowing of the blood flow and pallor of the gland was observed during each period of stimulation; 123 grams of blood was obtained in seven minutes (18.0 grams per minute). A specimen of ordinary venous blood was obtained from the right external jugular vein (D) and arterial blood from the femoral artery (E).

The thyroid lobes weighed 2 grams each. A specimen of each lobe was saved for histological examination and the rest was desiccated and powdered and preserved for the feeding experiments, and iodine determinations. No detectable iodine was found in these glands.

The blood specimens, after clotting were placed with the thyroid lobes into the drying oven at 55°C. When completely dried they were powdered and preserved for iodine determinations and feeding experiments. No detectable iodine was found in the quantities of blood available for testing.

Histological examination of the thyroid lobes revealed marked hyperplasia.

Protocol—Dog 3

February 12, 1918. Adult female; weight 9.125 kgm. Thyroid glands palpable. Anesthetised with ether and exposed both thyroid lobes; isolated the left vago-sympathetic nerve and separated the sympathetic from the vagus; attached guarded electrodes on the sympathetic nerve (without cutting the nerve). Inserted an oiled cannula into the vein at the upper pole of the left thyroid lobe and clipped off the vein at the lower pole. During off and on stimulation of the intact cervical sympathetic nerve, collected thyroid blood (A). The characteristic slowing of the blood flow was observed with each period of stimulation; 69.4 grams of blood was obtained in twenty minutes (3.46 grams per minute). Clotting in the cannula occurred and another cannula was inserted into the vein of the other lobe at the lower pole (the vein at the upper pole was not clipped off). A specimen of blood was collected without stimulation of the sympathetic (B): 10.1 grams of blood was obtained in four minutes (2.5 grams per minute). At the end of the collection of this specimen the blood in the cannula clotted; a cut was made in the vein near the cannula and blood allowed to run down alongside the cannula into a dish, after section of the vago-sympathetic nerve on this side (C): 33.8 grams of blood was obtained in ten minutes (3.4 grams per minute). In collecting the blood by this

means it was necessary to raise the gland and manipulate it frequently to facilitate the blood flow, thereby causing considerable massage of the gland during the collection of this specimen of blood. An indifferent blood specimen was obtained from the femoral artery (D).

The left thyroid lobe weighed 13.0 grams and contained 1.68 mgm. of iodine per gram of dried gland. The right lobe weighed 13.4 grams and contained 1.52 mgm. of iodine per gram of dried gland. A small piece of each gland was saved for histological examination and the rest was desiccated and powdered for the feeding experiments and iodine determinations.

The blood specimens, after clotting, were placed with the thyroid lobes into the drying oven at 55°C. When completely dried they were powdered and preserved for iodine determinations and feeding experiments. No detectable iodine was found in the quantities of specimens A and B available for testing; of specimen C 6 grams of the dried blood was available for iodine determination and in this specimen there was a detectable trace of iodine (somewhat less than 0.03 mgm. iodine in 6 grams of the dried blood).

Histological examination of the thyroid lobes showed the glands to be well stored with colloid material.

CHEMICAL TESTS FOR IODINE

After incinerating with sodium hydroxide, the different blood and thyroid gland specimens were tested for the presence of iodine by the method of Fresenius. In the quantities of blood that were available (0.4 to 18 grams) for testing, no detectable iodine was found in any of the bloods except specimen C of dog 3, which was collected during massage of the thyroid. This specimen gave a reaction equivalent to less than 0.005 mgm. of iodine per gram of dried blood.* The left lobe of dog 3 contained 1.68 mgm. of iodine per gram of dried gland and the right lobe contained 1.52 mgm. of iodine per gram of dried gland. The thyroid lobes of dogs 1 and 2 contained no detectable iodine.

*In another dog blood collected from the left lobe during sympathetic stimulation, with a flow of 1.43 gm. per minute, and another specimen collected from the right lobe during massage (after section of the right vago-sympathetic) with a flow of 19.4 gm. per minute gave negative iodine reactions. Tadpoles were not available. There was a large adenomatous tumor in the right lobe. The left lobe weighed 4.5 gm. and contained 1.81 mg. iodine per gram of dried gland. The right lobe weighed 7 gm. and contained 0.69 mg. iodine per gram of dried gland.

RESULTS OF FEEDING THYROID BLOOD TO TADPOLES

As in our previous work, the feeding was carried out in enamel-ware dishes each containing five tadpoles of uniform size in

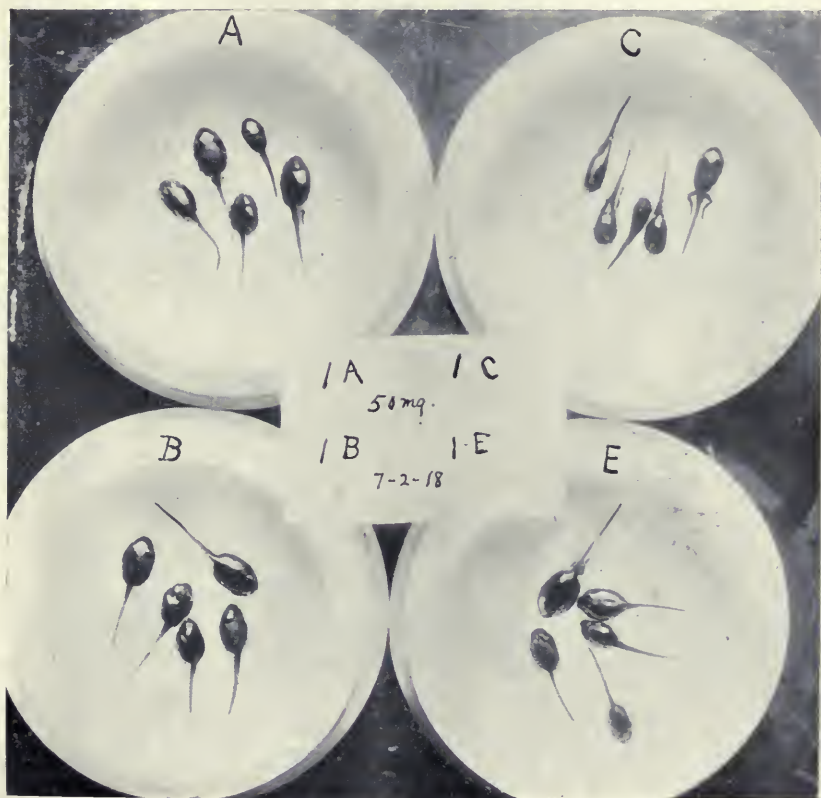


FIG. 1. BLOODS OF DOG 1 (SERIES I); FED IN DOSES OF 50 MGM. EVERY OTHER DAY FROM MAY 31 TO JULY 2

A, Thyroid blood, collected without stimulation of the vago-sympathetic; B, thyroid blood, collected during stimulation of the vago-sympathetic; C, thyroid blood, collected after cutting the vago-sympathetic; D, indifferent blood, obtained from the thyroid artery.

ordinary tap water. The substances to be tested were given every other day and fresh liver on the alternate days. The water was changed twice daily. The control tadpoles were given liver

only, every day. Two series were observed, the first series with tadpoles whose bodies were about 6 mm. in length and the second series with smaller tadpoles (about 3 to 4 mm. long). In the first series the desiccated bloods were offered in doses of 50, 100, and 200 mgm. and in the second series in doses of 75 and 100 mgm. The desiccated thyroid lobes were given in doses of 25 mgm. in both series. The feeding experiments were completed before the histological examination of the glands was made.

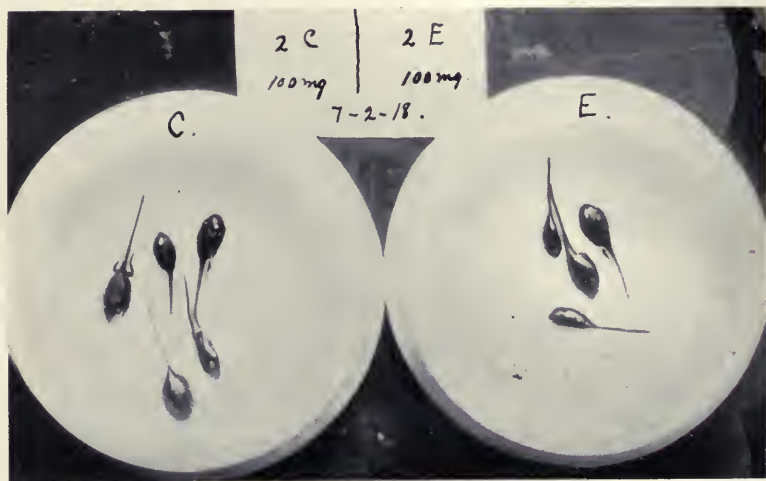


FIG. 2. BLOODS OF DOG 2, (SERIES 1), FED IN DOSES OF 100 MGM. EVERY OTHER DAY FROM MAY 31 TO JULY 2

C, Thyroid blood, collected during stimulation of the vago-sympathetic; E, indifferent blood, obtained from the femoral artery.

Although it seemed at first that all of the thyroid bloods caused some retardation of growth when compared with the effect of the ordinary (venous or arterial) blood, this effect was not sufficiently marked to be certain (figs. 1 and 2) except in the tadpoles getting the bloods from dog 3 collected during stimulation of the cervical sympathetic nerve, and during massage of the thyroid lobe (specimens A and C), which in both series caused definite uniform emaciation (figs. 3 and 4). Two important cor-

relative observations are associated with the activity of the bloods of dog 3, (1) the blood flow through the thyroid in this animal was considerably slower than in the other two dogs, and (2) the thyroid glands of dog 3 were normal and contained a good



FIG. 3. BLOODS OF DOG 3, (SERIES I); FED IN DOSES OF 100 MGM. (UPPER SET) AND 50 MGM. (LOWER SET) EVERY OTHER DAY FROM MAY 31 TO JULY 2

A, Thyroid blood, obtained during stimulation of the cervical sympathetic nerve; D, indifferent blood, obtained from the femoral artery.

store of iodine, while the glands of the other two animals were hyperplastic and contained no detectable iodine. The activity of the blood obtained during stimulation of the sympathetic nerve is not necessarily due to the existence of secretory nerves

to the thyroid. For the slowing of the blood flow due to vasoconstriction when this nerve is stimulated may result in a higher concentration of the internal secretion of the thyroid in the blood coming from the gland if the rate of liberation remains steady.



FIG. 4. BLOODS OF DOG 3, (SERIES II); FED IN DOSES OF 75 MGM. EVERY OTHER DAY FROM JUNE 8 TO JULY 2

A, Thyroid blood, obtained during stimulation of the cervical sympathetic nerve; B, thyroid blood, obtained without stimulation of the sympathetic nerve; C, thyroid blood, obtained during massage of the gland; D, indifferent blood, obtained from the femoral artery.

The best example of such a condition is afforded by the adrenal gland in which it has been shown that the concentration of epinephrin in the blood coming from the gland, in general, varies inversely with the rate of blood flow through the gland (9).

The activity of the thyroid lobes corresponded with their histological appearance and their iodine contents. The lobes of dogs 1 and 2 which were hyperplastic and contained no detectable iodine caused practically no effect upon the tadpoles, while the lobes of dog 3 which were well stored with colloid material and



FIG. 5. THYROID LOBES OF DOGS 1, 2, AND 3 (SERIES II); FED IN DOSES OF 25 MGM. EVERY OTHER DAY FROM JUNE 8 TO JULY 2

had a good content of iodine caused very marked emaciation and augmented differentiation (fig. 5).

Figure 6 shows the control tadpoles for series 1 and 2. These tadpoles were given only liver (daily) and were used as controls for the tadpoles getting the indifferent bloods which were to be compared with those getting the thyroid blood.

All of the figures except figure 7 are reduced to $\frac{4}{5}$ of the actual size.

Figure 7 shows the histological structure of the thyroid glands of dogs 1, 2, and 3.



FIG. 6. CONTROLS. (SERIES I AND II); FED WITH FRESH LIVER ONLY, EVERY DAY

Series I, from May 31 to July 2; series II, from June 8 to July 2.

It is interesting to note that massage of the thyroid gland apparently is capable of liberating the active material from the gland into the blood. This also is in accord with what has been shown to occur in the adrenal under similar conditions (10, 11). It would not be profitable to speculate as to whether the histological condition of the gland or the slow blood flow resulting in

a high concentration of active material in the blood coming from the thyroid in dog 3 is the more important factor in the result obtained on tadpoles with specimen A. Concentrating the active material in the thyroid blood might be a means of obtaining quantitative information on the rate of liberation of the thyroid secretion. If the active material in the thyroid blood is the same as that in the gland, it is probable that concentration could be effected by the method of alkaline hydrolysis employed by Kendall (12). As tadpoles are available during a certain period of the year only, this and other suggestions have been reserved for future study.

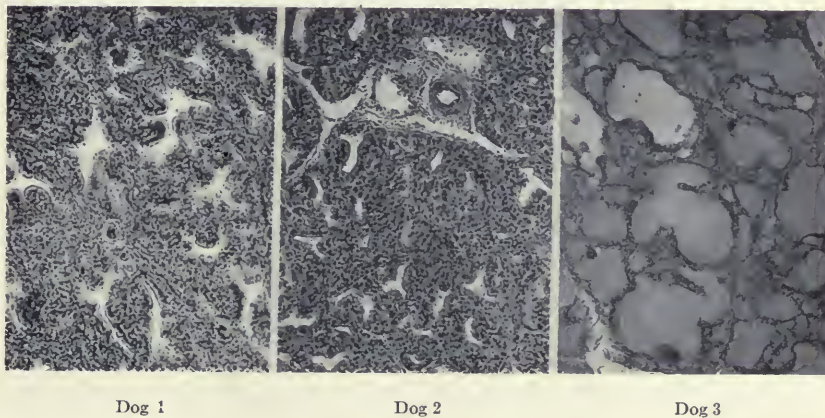


FIG. 7. PHOTOMICROGRAPH OF SECTIONS OF THYROID OF DOGS 1, 2, AND 3 ($\times 33$)

SUMMARY

1. An attempt was made to detect in the blood coming from the thyroid glands of three dogs, a physiologically active secretion, by feeding the dried blood to tadpoles.

2. One dog, whose thyroid glands were rich in colloid and had a good iodine content, yielded evidence of an active secretion into the blood collected from the glands during massage and during stimulation of the cervical sympathetic nerve. As indicated in the text this result yields no evidence of the existence of secretory nerves to the thyroid for it is not possible to know the rate of liberation of the secretion, and an increased concentration of

the secretion in the thyroid blood alone can not be taken as evidence of increased liberation.

3. Two dogs whose thyroid glands were hyperplastic and contained no detectable iodine yielded negative results.

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NOTE ON THE PREPARATION OF A SOLUBLE CONCENTRATED PRODUCT OF THE THYROID GLAND

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During the course of the investigation reported in the preceding paper, the opportunity was available to test the effect on tadpoles of a product of thyroid which was obtained in an attempt to extract a concentrated water-soluble compound. The product "A" obtained by alkaline hydrolysis of normal thyroids of hogs, according to Kendall's method (1), was subjected to further hydrolysis in water acidified with hydrochloric acid. When the substance was completely digested the resulting solution was filtered through a Chamberland filter. To the clear filtrate was added hydrated aluminum silicate (Lloyd's Reagent) and the mixture thoroughly shaken and filtered through paper. The hydrated aluminum silicate on the filter was thoroughly washed with water until all of the acid was washed out. The adsorbed product was now separated by slowly percolating through the material on the filter a dilute solution of ammonia in water until the percolate came through entirely colorless. The ammoniacal solution was then heated on a water bath and the ammonia driven off with the aid of a current of air passed through the solution. The resulting aqueous solution was reddish brown and on evaporation yielded an amorphous powder. This powder contained 13.44 mgm. of iodine per gram of dry substance and the product "A" from which it was obtained contained 16 mgm. per gram. A small quantity of the product was available for feeding experiments with tadpoles. This product showed very nearly the same degree of activity as the product "A" from which it was obtained. Both products were given to the tadpoles in

doses of 0.5 and 1 mgm. every other day. They caused extreme emaciation and differentiation. In the tadpoles getting 1 mgm. doses the effect was produced so rapidly that very little difference could be observed in the activity of the two products. The tadpoles getting 0.5 mgm. doses indicated a slight difference in activity in favor of the product "A." No attempt was made to determine whether the slightly greater activity of the product

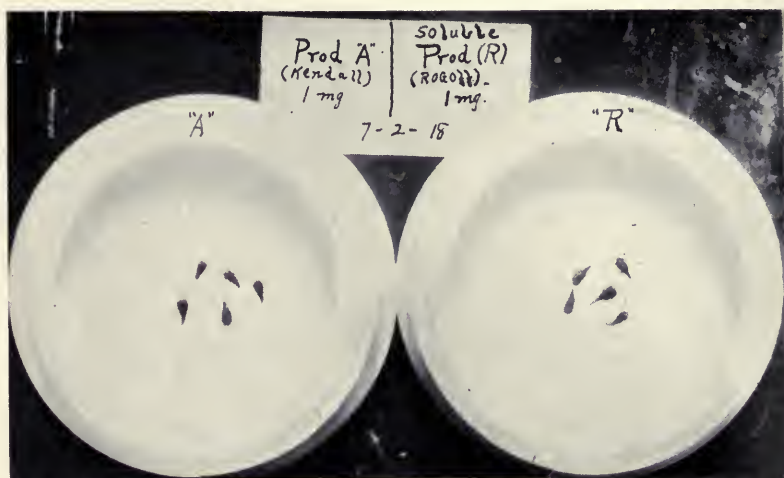


FIG. 1. PRODUCT "A" (KENDALL) (LEFT); SOLUBLE PRODUCT (ROGOFF) (RIGHT)

Fed in doses of 1 mgm. every other day from June 8 to July 2 (for controls, see figure 6, series II, in the preceding paper). Reduced to $\frac{1}{4}$.

"A" was proportional to the higher iodine content, as there was not sufficient material available for another series of experiments in which the effects of smaller doses could be observed and quantitative observations made.

Figure 1 shows the marked effect produced by both products when fed to tadpoles in doses of 1 mgm. every other day. The control tadpoles for this figure are shown in figure 6 (series II) in the preceding paper.

SUMMARY

The preparation of a concentrated active product of the thyroid gland, which is soluble in water, is briefly described.

REFERENCE

- (1) KENDALL, E. C.: A method for the decomposition of the proteins of the thyroid, with a description of certain constituents. *Jour. Biol. Chem.*, 1915, xx, 501.

HOMEOTRANSPLANTATION AND AUTOTRANSPLANTATION OF THE SPLEEN IN RABBITS.

III. FURTHER DATA ON GROWTH, PERMANENCE, EFFECT OF AGE, AND PARTIAL OR COMPLETE REMOVAL OF THE SPLEEN.

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(Received for publication, February 27, 1920.)

We have been unable to find any references in the literature to the transplantation of fragments of spleen to parts of the body widely separated from the normal neurovascular field of this organ in addition to those referred to in 1917. At that time^{1, 2} we reviewed the literature and reported our first experiments with spleen homeografts and autografts in fifteen rabbits. The present paper includes the data of further experiments with homeografts and with autografts, together with certain general physiological reactions relative to the spleen which this study has emphasized.

Method.

The method consists of transferring small fragments of the spleen roughly 2 by 2 mm. to the subcutaneous tissues of the abdomen under strict aseptic precautions. The tissue to be transplanted was kept in isotonic salt solution at temperatures varying between 36° and 39°C. Sometimes the tissue was transplanted within a few minutes after removal, while in others it was kept in salt solution as long as 3 hours. The fragment to be transplanted was rinsed in the salt solution, but no other attempt to remove contained blood was made.

¹ Manley, O. T., and Marine, D., The transplantation of splenic tissue into the subcutaneous fascia of the abdomen in rabbits, *J. Exp. Med.*, 1917, xxv, 619.

² Marine, D., and Manley, O. T., Influence of age on the permanence of subcutaneous autografts of the spleen in rabbits, *Proc. Soc. Exp. Biol. and Med.*, 1916-17, xiv, 123.

After making a transverse abdominal skin incision approximately 2 cm. in length, usually one on each side of the umbilicus, the subcutaneous fascia was lifted with fine forceps and an area free from blood vessels was punctured with a cataract knife, the tissue introduced, and the fascial opening closed with a ligature. The skin incision was sutured and sealed with celloidin. All operative procedures were carried out under ether anesthesia. All subsequent examinations were direct (autopsy or operation). If the examination was made by operation, ether anesthesia was used, and with aseptic precautions the skin was incised, the graft or its site was exposed, measurements were taken, the graft was removed for microscopic examination or left *in situ*, and the skin closed with suture and sealed with celloidin. The intervals between examinations are given in the tables. This technique has been employed in all our transplantation work with ductless gland tissues.

Both homeografts and autografts were studied in relation to taking and growth or regeneration, in relation to age of the animals, and in relation to partial and complete removal of the spleen and to permanence of the grafts.

Homeotransplantation.

The data of the experiments are given in Table I. Only the rabbits which had not been subjected to previous homeografts of any tissue are included in the table. This was done to avoid the untoward effect of active immunity which such grafts produce and to render conditions as favorable as possible for taking and growth. Notwithstanding this important precaution not a single graft was found active after the 30 day period, a period arbitrarily chosen, but somewhat longer than the time necessary for the full development and destructive effect of the active immunity. The experiments were not studied from the standpoint of determining how rapidly the grafts were destroyed, though by comparison they show very clearly that the destruction of spleen is much more rapid than that of thyroid tissue transplanted under the same conditions. Thus in a large number of routine homeotransplants of thyroid we have obtained about 10 per cent active at the end of 30 days, though ultimately (in the course of

60 to 90 days) these also might be destroyed. These observations indicate that spleen tissue produces a high degree of immunity more quickly than does thyroid.

The well established favorable factor of blood relation in the transplantation of certain tissues was also utilized in the hope that grafts might survive beyond the 30 day period, but with spleen we were unsuccessful. The antigenic power of spleen, then, is very high and on a level with that of other hematopoietic tissues. In a few instances in which the grafts were examined at the 7th, 8th, and up to the 16th day, active splenic tissue, well established blood supply, and evidence of growth were found. This is observed generally with homeografted tissues. Many literature reports of positive homeografts of tissues are misleading because they have emphasized or reported only this early stage. Effective immunity to foreign proteins in general does not become manifest until the 8th to 10th day, as shown by studies on anaphylaxis. Within this period homeografts usually take and often show growth, while after this period rapid destruction takes place. Different tissues show different rates of destruction. In our experience lymphoid (lymph gland and thymus) and splenic tissues undergo destruction more rapidly than thyroid, and thyroid cells more rapidly than cells rich in lipoids, as adrenal cortex and interstitial cells of the ovary and testis. Associated with this destruction there is the well known infiltration or invasion of the graft with lymphoid cells. Much importance has been assigned to this phenomenon and some authors, notably Murphy and his coworkers,³ have claimed for it a primary importance in the death and destruction of homeografts. We cannot subscribe to this view. We believe that the primary injury is due to the action of antibodies and that the lymphoid cells are attracted to the site secondarily, and in response to a special low grade irritation which injured cells set up. Loeb⁴ and Sittenfield⁵ also have expressed the view that lymphocytes are not primarily concerned in the destruction of foreign proteins.

³ Murphy, Jas. B., and Morton, J. J., The lymphocyte in natural and induced resistance to transplanted cancer. II, *J. Exp. Med.*, 1915, xxii, 204.

⁴ Loeb, Leo, Multiple transplantations of the thyroid and the lymphocytic reaction, *J. Med. Research*, 1918-19, xxxix, 71.

⁵ Sittenfield, M. J., The significance of the lymphocyte in immunity to cancer, *J. Cancer Research*, 1917, ii, 151.

TABLE I.
Homeotransplantation of Spleen.

Rabbit No.	Series No.	Age.	Sex.	Date. Splenectomy.	Date transplanted. Spleen used.		First examination.	Final examination.	Additional data.
					R.*	L.			
		days		1917	1917	1917			
1	4-46	26	M.	Jan. 18 Complete.	Jan. 18 3-155	Jan. 18 3-155		L. 54-	Jan. 18. L. adrenalectomy. Thyroids and thymus enlarged. Thymus atrophic.
2	4-48	33	F.	Jan. 25 Complete.	Jan. 25 3-157	Jan. 25 3-157	L. 47±†	" 132-	Jan. 25. L. adrenalectomy; homeo-graft of adrenal.
3	4-52	40	M.	Feb. 1 Complete.	Feb. 1 3-159	Feb. 1 3-159	R. 40-	R. 125-	Feb. 1. L. adrenalectomy. Thymus present; thyroid large.
4	4-54	47	"	Jan. 30 Complete.	Jan. 30 3-161	Jan. 30 3-161		" 42-	Jan. 30. L. adrenalectomy; homeo-graft of adrenal.
5	4-64	54	F.	Feb. 6 Complete.	Feb. 6 3-163	Feb. 6 3-163	R. 35-	" 121-	Thymus large; thyroid normal. Feb. 6. L. adrenalectomy.
6	4-74	61	"	Feb. 13 Complete.	Feb. 13 3-165	Feb. 13 3-165	" 58-	" 114-	Thymus atrophic; thyroid normal. Feb. 13. L. adrenalectomy; homeo-graft of adrenal.
7	4-94	90	M.	Mar. 22 Complete.	Mar. 22 Complete.	June 7 3-184		L. 98-	Thymus large; thyroid normal. Mar. 22. L. adrenalectomy.
8	4-17	123	"	Dec. 3 Complete.	Dec. 3 Complete.	Dec. 16 1-320		" 67-	Thymus cellular; thyroid normal. Spleen was enlarged; thymus small; adrenals small.
9	4-78	168	"	Feb. 15 Complete.	Feb. 15 Complete.	Feb. 15 3-169		" 56-	Dec. 3. Thyroidectomy. Feb. 15. L. adrenalectomy. Chronic nephritis; thymus atrophic; thyroid and parathyroids enlarged.

10	4-2	Adult.	M.	1915 Aug. 26 Complete.	1915 Nov. 29 1-313	L. 15—	Nov. 29. Thyroidectomy.
11	4-3	"	F.	Aug. 26 Complete.	Dec. 16 1-320	" 65—	" 29.
12	4-4	"	M.	1915 Dec. 16 Nov. 5 Complete.	Nov. 29 1-313	R. 67— L. 17—	" 29.
13	4-5	"	"	Nov. 5 Complete.	Dec. 18 1-321	R. 63—	Dec. 2.
14	4-6	"	F.	May 11 Complete.	Dec. 2 1-315	L. 14±(Dec. 2). " 65—(" 16).	" 2.
15	4-7	"	M.	Nov. 5 Complete.	Dec. 16 1-320	L. 65—	" 2.
16	4-8	"	"	Nov. 5 Complete.	Dec. 16 1-320	" 67—	" 3.
17	4-9	"	F.	Nov. 5 Complete.	Dec. 16 1-320	" 119—	" 3.
18	4-42	"	"	1916 Dec. 12 Complete.	Dec. 12 3-151	" 43—	" 12. " L. adrenalectomy.
19	4-44	Old adult.	M.	Dec. 19 Complete.	1917 Feb. 20 1-411	R. 23—	Dec. 12, 1916. Thyroidectomy; L. adrenalectomy.
20	4-93	52	F.	1917 Mar. 20 ½ cm. Apr. 17 1 cm.	Apr. 17 3-179	L. 52—	Mar. 20. L. adrenalectomy. Thymus atrophic; thyroid normal.

* R. indicates right; L., left.

† The condition of the transplants at the different examinations is expressed by plus or minus signs instead of the actual measurements obtained in order to facilitate comparison. The figures express the intervals in days.

TABLE I—Continued.

Rabbit No.	Series No.	Age.	Sex.	Date. Splenectomy.	Date transplanted. Spleen used.		First examination.	Final examination.	Additional data.
					R.*	L.			
21	4-104	days 80	M.	1917 Apr. 17 $\frac{1}{4}$ cm.	1917	1917 Apr. 17 3-179	L. 53—	L. 163—	All organs intact; same litter as Nos. 4-105 and 4-106; thyroid slightly enlarged; thymus present. All organs intact.
22	4-105	80	"	Apr. 17 1 cm.		Apr. 17 3-179	L. 53—	" 53—	" " " thymus small.
23	4-106	80	"	Apr. 17 1 cm.		Apr. 17 3-179	L. 53—	" 163—	" " " " "
24	4-108	80	"	Apr. 17 $\frac{3}{4}$ cm.		Apr. 17 3-192	L. 58±	" 52—	" " " " "
25	4-98	104	F.	Apr. 10 $\frac{1}{4}$ of spleen.		Apr. 10 3-177	" 169—	" 169—	Thyroid lobes large, vascular; thymus normal.
26	4-101	60	M.		Apr. 10 3-177		R. 56—	R. 56—	June 26, 1916. R. adrenalectomy. Oct. 10. Gonadectomy. Mar. 13, 1917. Thyroidectomy.
27	4-65	mos. 4	"			Feb. 6 3-163		L. 23—	Spleen small; thymus very large. Feb. 6. Thyroidectomy; much enlarged; H ₂ SO ₄ .
28	4-49	4	F.			Jan. 25 3-158		" 21—	Parathyroids very large; thymus large.
29	4-50	4	"			Jan. 25 3-157		" 40—	Jan. 25. Thyroidectomy; H ₂ SO ₄ . " 25. " H ₂ SO ₄ .
30	4-55	4	"			Jan. 30 3-162	R. 14++	R. 35—	Spleen small; parathyroids very large. Jan. 30. Thyroidectomy; H ₂ SO ₄ . Spleen enlarged; parathyroids very active.

31	4-56	4	F.	Jan. 30 3-162			R. 30-	Jan. 30. Thyroidectomy; H ₂ SO ₄ . Spleen normal; thymus active.
32	4-57	4	"	Jan. 30 3-162			" 30-	Jan. 30. Thyroidectomy; H ₂ SO ₄ . Adrenals and spleen normal; thymus atrophic.
33	4-58	4	M.	Jan. 30 3-162			" 30-	Jan. 30. Thyroidectomy; KOH.
34	4-59	4	"	Feb. 1 3-159			" 28-	Feb. 1. " H ₂ SO ₄ . Spleen and thymus normal.
35	4-60	4	"	Feb. 6 3-159			" 28-	Feb. 6. Thyroidectomy; H ₂ SO ₄ and KOH.
36	4-61	4	F.	Feb. 6 3-163			L. 23+	Spleen small; thymus very large.
37	4-76	5	M.	Feb. 13 3-166		L. 16+	" 42-	Feb. 6. Thyroidectomy; H ₂ SO ₄ . Thymus atrophic.
38	4-62	5	F.	Feb. 6 3-163			" 23-	Feb. 13. Thyroidectomy; KOH.
39	4-75	Adult.	"	Feb. 13 3-165		L. 16+	" 42-	" 6. " KOH.
40	4-81	"	"	Feb. 15 3-169			" 8+	" 13. " H ₂ SO ₄ and KOH.
41	4-82	"	"	Feb. 15 3-174			R. 42-	Jan. 23. Thyroidectomy.
42	4-99	"	M.	Apr. 10 3-177			" 7+	" 23. "
43	4-100	"	"	Apr. 10 3-177			" 58-	Sept. 28, 1916. R. adrenalectomy. Jan. 16, 1917. L. adrenalectomy. Mar. 3. Thyroidectomy; H ₂ SO ₄ . Thymus atrophic; spleen small. Sept. 22, 1916. R. adrenalectomy. Jan. 11, 1917. L. adrenalectomy. Mar. 13. Thyroidectomy. Thymus atrophic; spleen small.

TABLE I—*Concluded.*

Rabbit No.	Series No.	Age.	Sex.	Date. Splenectomy.	Date transplanted. Spleen used.		First examination.	Final examination.	Additional data.
					R.*	L.			
44	4-102	Adult.	M.		¹⁹¹⁷ Apr. 10 3-177			R. 7+	May 25, 1916. R. adrenalectomy. June 1. L. adrenalectomy. Dec. 7. Thyroidectomy; gonadectomy. Spleen small; thymus atrophic.
45	4-109	"	"		Apr. 10 3-177			" 58—	Sept. 26, 1916. R. adrenalectomy. Jan. 11, 1917. L. adrenalectomy. Mar. 13. Thyroidectomy. Thymus large; spleen normal; L. ad- renal much enlarged.
46	4-40	"	"		Jan. 25 3-158			" 26—	Dec. 26, 1916. Thyroidectomy; H ₂ SO ₄ .
47	4-21	"	"		¹⁹¹⁶ Dec. 19 3-154			L. 34—	Mar. 11, 1916. R. adrenalectomy. Dec. 19. L. adrenalectomy. Jan. 9, 1917. Gonadectomy. Thyroid normal; thymus very large.
48	4-39	"	"		Jan. 25 3-158			R. 26—	Jan. 2. Thyroidectomy.
49	4-43	"	"		Jan. 25 3-158			" 26—	" 2. " Previous homeographs.
50	4-87	Old adult.	F.		¹⁹¹⁷ Feb. 27 3-167			L. 23—	Jan. 22. Thyroidectomy. Many previous homeographs.

Effect of Age.—This factor has no appreciable influence. In this series rabbits of known age and parentage, varying from 26 to 168 days, as well as a large number of adults of different but known ages, were used. In no instance did the graft survive the 30 day period. Variations in the rate of destruction depending on age must be expected, but their detection in the case of spleen would require frequent examinations within the 30 day period. Further attempts were made to determine whether splenectomy, partial or complete, had any effect. No noteworthy difference in the outcome even in young rabbits was detected whether the spleen was intact, or partially or completely removed at the time of, before, or after transplantation. The experiments do not eliminate the possibility of an advantage accruing to the transplant from induced splenic insufficiency, but indicate that if splenectomy is of aid it must be looked for by frequent examinations within the first 30 days, since by that time the developed immunity has destroyed any possible evidence.

Thyroidectomy and partial or complete adrenalectomy combined or separate likewise have no noteworthy effect on delaying destruction of the grafts and, therefore, probably on the degree of immunity developed. In this connection it may be pointed out that Gates⁶ was unable to detect any noteworthy difference in the degree of immunity to sheep erythrocytes and typhoid bacilli obtained in guinea pigs with and without partial removal of the adrenal glands. We have found⁷ that thyroidectomy and splenectomy alone or combined have no marked effect on the antibody formation following injection of sheep erythrocytes in rabbits. Bullock and Rohdenburg⁸ find that splenectomy has no influence on the immunity to transplanted tumors.

⁶ Gates, F. L., Antibody production after partial adrenalectomy in guinea pigs, *J. Exp. Med.*, 1918, xxvii, 725.

⁷ Unpublished results.

⁸ Bullock, F. D., and Rohdenburg, G. L., Splenectomy exerts no appreciable influence upon immunity against transplanted tumors, *J. Cancer Research*, 1917, ii, 465.

Autotransplantation.

The data concerning these experiments are given in Tables II and III. Usually two fragments were transplanted, one to the right and the other to the left of the umbilicus, and designated as right and left transplants. In the six experiments reported in 1917 one failed and in the present series one failed. This was due to necrosis. Failure of autografts to take always indicates some gross technical error. Some of these rabbits had been used for homeotransplantation of other tissues—adrenal, thyroid, and sex glands. Previous or coincident homeografts have no demonstrable effect on the taking or growth of autografts. The subsequent course of these autografts was followed and it was found to be modified by several factors, the most striking of which are age and partial or total removal of the spleen.

Effect of Age.—The experiments have been arranged in Tables II and III according to the age at the time of transplantation. The youngest rabbit used was 26 days old and the oldest whose age was definitely known was 320 days; one other is listed as 586+ days because it had been in the laboratory for this length of time before being utilized for this purpose. A maximum of three examinations is given in the tables, although in several instances five, six, and more examinations have been made. A consideration of the series as a whole shows a distinct decrease in the amount of growth of the transplants as one passes from the youngest to the oldest rabbits of the series, although other factors remained as constant as possible. The greatest and most rapid growth of transplants occurred in the youngest rabbits, also the least growth in the oldest. While this decrease with age in the rate and amount of growth is gradual, the differences become striking about the 4th to 5th month. In our series the first instance of failure to obtain marked growth was in a rabbit 132 days old. The remaining ten rabbits, 167 or more days old at the time of transplantation, failed to show marked growth. This change in the rate of growth corresponds roughly with the time of sexual maturity or early adult life. There is a suggestion that in adult rabbits transplants that in the 1st month showed some growth tended to involute or undergo atrophy later. This tendency to atrophy or

involution was not seen in young rabbits with transplants of similar duration. As suggested in a previous note² two factors (possibly others) may be considered. The first is that growth of the transplants is a part of the normal growth of the animal and ceases when physical growth is complete. This possibility is disproved by the fact that such transplants fail to show marked growth even in the young if the spleen is not removed. The second factor would presuppose that the value of the spleen to the organism decreases with age and that after adult life whatever functions the organ normally has may be assumed by other tissues, possibly lymph glands and bone marrow. This view is supported by the results obtained by the removal of the spleen which follow.

Effect of Partial and Complete Removal of the Spleen on Autografts.

—The data of these fifteen experiments are given in Table III. The amount of spleen removed and the dates are given in the table. Usually less than 0.5 cm. of the anterior portion was removed at the time of transplantation.⁹ The youngest rabbit with partial removal of spleen and transplantation was 52 days old and the oldest 124 days. The same differences in the growth of transplants, dependent upon age and independent of amount of spleen removed, are noted as in Table II. Thus in No. 1, a 52 day old rabbit in which one-fourth of the spleen was removed, the transplants were ++ at 80 days and +++ at 177 days, while in No. 15, a 124 day old rabbit in which one-eighth of the spleen was removed, no growth took place in 132 days. Between these extremes there is the same evidence of gradation of growth dependent upon age as shown in Table II. There is no instance of a very young rabbit with a minimum amount of spleen removed. Such experiments would show whether in the absence of splenic deficiency marked growth could occur. This question, however, is clearly answered in the negative for 52 day old rabbits by Experiments 1, 2, and 3, in which all were from the same litter. Two had about one-fourth of the spleen removed at the time of transplantation and at the first examinations 80 and 52 days later both were ++, while the third, which had only one-eighth of the

⁹ There are considerable variations in the size of the spleen in apparently healthy rabbits, just as in other animals, which cannot be accounted for with our present knowledge of physiology and pathology.

TABLE II.
Autotransplantation of Spleen (Complete Removal of Spleen).

Rabbit No.	Series No.	Age.	Weight.	Sex.	Date of splenectomy (complete).	Date transplanted.	First examination.	Second examination.	Final examination.	Additional data.
		days	gm.		1917	1917				
1	4-45	26	330	M.	Jan. 18	Jan. 18	R. 54+++ L. 54+++	R. 145+++ L. 145+++	R. 278+++ L. 278+++	Jan. 18. L. adrenalectomy. May 1. Thyroidectomy.
2	4-46	26	315	"	" 18	" 18	R. 54+++ " 47++	R. 139+++ " 96+++	R. 141+++ " 230+++	Jan. 18. L. adrenalectomy. " 25.
3	4-47	33	575	"	" 25	" 25	L. 47++	L. 96+++	L. 230+++	H ₂ SO ₄ . May 1. Thyroidectomy.
4	4-48	33	505	F.	" 25	" 25	R. 47++	R. 132+++	R. 134+++	Jan. 25. L. adrenalectomy.
5	4-51	40	330	"	Feb. 1	Feb. 1	" 40++ L. 40++	" 110+++ L. 110+++	" 224++ L. 224++	Feb. 1. " " H ₂ SO ₄ . May 1. Thyroidectomy; transplants slightly decreased.
6	4-52	40	415	M.	" 1	" 1	" 40++	" 125++	" 232+++	Feb. 1. L. adrenalectomy. Thyroids markedly enlarged at autopsy.
7	4-53	47	405	F.	Jan. 30	Jan. 30	R. 42++ L. 42++	R. 226+++ L. 226+++	R. 384+++ L. 384+++	Jan. 30. L. adrenalectomy; H ₂ SO ₄ . May 1. Thyroidectomy.
8	4-63	54	410	"	Feb. 6	Feb. 6	R. 35++ L. 35++	R. 84+++ L. 84+++	R. 121+++ L. 121+++	Feb. 6. L. adrenalectomy; KOH. May 1. Thyroidectomy.
9	4-64	54	310	"	" 6	" 6	" 35++	" 121++	" 133++	Feb. 6. L. adrenalectomy. Thyroids small.

10	4-73	61	555	F.	Feb. 13	R. 58++++ L. 58++++	R. 77++++ L. 77++++	R. 119++++ L. 119++++	Feb. 13. L. adrenalectomy; KOH. May 1. Thyroid- ectomy.
11	4-94	61	595	"	" 13 1916	" 58++++	" 114++++	" 115++++	Feb. 13. L. adrenalectomy. Thyroid normal.
12	4-38	66	1,275	M.	Dec. 7 1916	" 36+	" 82++	" 183++	Dec. 6. L. adrenalectomy; phosphoric acid.
13	4-37	66	875	F.	" 7	" 36+	" 56++	" 82++	Dec. 6. L. adrenalectomy; phosphoric acid.
14	4-36	66	1,225	M.	" 7	" 36+	" 56+	" 181++	Dec. 6. L. adrenalectomy; phosphoric acid. Feb. 27, 1917. Thyroidectomy.
15	4-86	75	790	"	1917 Feb. 27	R. 44++ L. 44++	R. 63++++ L. 63++++	R. 197++++ L. 197++++	Feb. 27. L. adrenalectomy; KOH. May 1. Thyroidec- tomy.
16	4-26	83	1,620	"	1916 Dec. 5	" 38++	" 290++++	" 1,181++++	Dec. 5. L. adrenalectomy; phosphoric acid; double li- gation of vas deferens. Apr. 5, 1917. L. gonadectomy; partial thyroidectomy.
17	4-25	83	1,450	F.	" 5	" 38+	" 84++++	" 185++++	Dec. 5. L. adrenalectomy; phosphoric acid; partial thy- roidectomy.
18	4-110	84	1,050	"	1917 May 8	R. 31++ L. 31++	R. 141++++ L. 141++++	R. 679++++ L. 679++++	Pregnant; thyroids normal.

TABLE II—Concluded.

Rabbit No.	Series No.	Age.	Weight.	Sex.	Date of splenectomy (complete).	Date transplanted.	First examination.	Second examination.	Final examination.	Additional data.
		days	gm.		1917	1917				
19	4-112	84	1,090	M.	May 8	May 8	R. 31+ L. 31+		R. 141+ L. 141+	Thyroids normal; lack of growth of transplants; large accessory spleen.
20	4-113	84	1,225	"	" 8	" 8	R. 31+ L. 31+	R. 141+ L. 141+	R. 475+ L. 475+	Example of acute enlargement of spleen transplant with pneumonia. White blood corpuscles 30,800.
21	4-94	90	1,195	"	Mar. 22	Mar. 22	R. 77+ L. 77+		R. 175+ L. 175+	Mar. 22. L. adrenalectomy.
22	4-95	97	1,200	F.	Apr. 3	Apr. 3	R. 64+ L. 64+	R. 176+ L. 176+	R. 1,062+ L. 1,062+	
23	4-97	104	1,265	M.	" 10	" 10	R. 58+ L. 58+		R. 169+ L. 169+	Spleen much enlarged at time of removal; evidence that transplants grew more rapidly, then decreased with recovery of animal.
24	4-103	111	1,275	"	" 17	" 17	R. 51+ L. 51+		R. 163+ L. 163+	
25	4-119	132	2,240	"	June 7	June 7			R. 111+ L. 111+	Failure to obtain growth with spleen removed.
26	4-77	167	3,025	"	Feb. 15	Feb. 15	R. 56+ L. 56+	R. 75+ L. 75+	R. 209+ L. 209+	Feb. 15. L. adrenalectomy; KOH. May 1. Thyroidectomy.

27.	4-78	167	3,445	M.	Feb. 15	Feb. 15	R. 56-		R. 113-	Feb. 15. L. adrenalectomy; enlarged thyroid lobes. Failure to take probably due to error in technique.
28	4-84	173	2,575	F.	" 22	" 22	" 49+	R. 68+	" 106+	Feb. 22. L. adrenalectomy; KOH; thyroidectomy.
29	4-85	173	3,425	"	" 22	" 22	L. 49+	L. 68+	L. 106+	Feb. 22. L. adrenalectomy; KOH.
30	4-79	320	4,835	"	" 15	" 15	R. 49+	R. 68+	R. 202+	Died; pneumonia.
31	4-80	320	4,435	"	" 15	" 15	R. 56++		" 61++	"
32	4-83	320	2,940	M.	" 20	" 20	L. 56++		L. 61++	"
33	4-68	396	2,525	"	" 8*	" 8	R. 51+	R. 114+	R. 23+	Feb. 20. L. adrenalectomy; thyroidectomy. Apr. 3. Gonadectomy.
34	4-71	396	2,790	"	" 8	" 8	" 33+	L. 114+	L. 216+	June 22, 1916. R. adrenalectomy. Oct. 10. L. gonadectomy. Apr. 11, 1916. Thyroidectomy.
35	4-72	586+	3,150	"	" 8	" 8	R. 33+		R. 118+	Feb. 12, 1916. Thyroidectomy. Nov. 3. R. adrenalectomy.
							L. 33+		L. 118+	Apr. 5. Gonadectomy. Oct. 25, 1915. Thyroidectomy.

* Small accessory spleen left.

TABLE III.
Autotransplantation of Spleen.

Rabbit No.	Series No.	Age.	Weight.	Sex.	Date of splenectomy.	Date transplanted.	First examination.	Second examination.	Final examination.	Additional data.
		days	gm.		1917	1917				
1	4-92	52	720	M.	Mar. 20. ½ removed.	Mar. 20	R. 80++ L. 80++		R. 177++++ L. 177++++	Mar. 20. L. adrenalectomy. Thyroid normal.
2a	4-93	52	700	F.	Mar. 20. ¼ removed.	" 20	R. 52++ L. 52++		R. 177++++ L. 177++++	Mar. 20. L. adrenalectomy. Thyroid normal.
b					Apr. 17. ¼ removed.	Apr. 17	R. 52++		R. 150++	
3a	4-90	52	635	M.	Mar. 20. ¾ removed.	Mar. 20	" 80+ L. 80+		" 177++++ L. 177++++	Mar. 20. L. adrenalectomy. Very little growth in first 80 days; marked after removal of spleen; compensatory.
b					June 9. Remainder removed.					
4a	4-88	64	1,050	"	Mar. 20. ¾ removed.	Mar. 20	R. 49+ L. 49+		R. 177++++ L. 177++++	Mar. 20. L. adrenalectomy. Thyroid slightly enlarged; very little growth in first 80 days; marked after removal of spleen.
b					May 8. Remainder removed.			R. 79++++ L. 79++++		
5a	4-89	64	985	"	Mar. 20. ¾ removed.	Mar. 20	R. 49+ L. 49+		R. 177++++ L. 177++++	Mar. 20. L. adrenalectomy. Thyroid vascular; slightly enlarged. Rapid growth after removing spleen.
b					May 8. Remainder removed.			R. 79++ L. 79++		

6	4-104	80	960	M.	Apr. 17. $\frac{1}{2}$ removed.	Apr. 17	R. 53+	R. 163+	No growth with spleen intact.
7	4-105	80	1,055	"	Apr. 17. $\frac{1}{2}$ removed.	" 17	" 53+	" 196+	No growth with spleen intact.
8	4-106	80	1,030	"	Apr. 17. $\frac{1}{2}$ removed.	" 17	" 53+	" 163+	No growth with spleen intact.
9	4-114	84	1,260	F.	May 8. $\frac{1}{2}$ removed.	May 8	" 31+	" 141+	No compensatory growth of transplants.
10	4-115	84	1,075	"	May 8. $\frac{1}{2}$ removed.	" 8	R. 31+ L. 31+	R. 141+ L. 141+	Thyroid vascular and enlarged.
11	4-107	87	1,110	"	Apr. 24. $\frac{1}{2}$ removed.	Apr. 24	R. 52+ L. 52+	R. 163+ L. 163+	Failure to obtain growth without spleen removal.
12	4-108	87	1,010	M.	Apr. 24. $\frac{1}{2}$ removed.	" 24	R. 52+	R. 198+ (very small).	Failure to obtain growth without spleen removal.
13	4-96	97	1,245	"	Apr. 3. $\frac{1}{2}$ removed.	" 3	" 64+ L. 64+	" 176+ R. 176+	No compensatory hyperplasia.
14	4-98	104	985	F.	Apr. 10. $\frac{1}{2}$ removed.	" 10	R. 58+ L. 58+	R. 169+ L. 169+	Decreased with recovery of animal. Partial removal failed to produce growth.
15	4-118	124	1,625	M.	May 17. $\frac{1}{2}$ removed.	May 17	R. 21+ L. 21+	R. 132+ L. 132+	Failure to obtain growth of transplant with spleen intact.

spleen removed, was only + at the first examination 80 days later. The remainder of the spleen in this rabbit was then removed and at 177 days the transplants were + + + +, while the first two which did not have the remainder removed were only + + + at 177 days. The same reaction is seen in Experiments 4 and 5, little if any growth occurring until the spleen was removed, when a marked growth promptly took place. The effect of splenic deficiency in stimulating the growth of transplants is striking, as is shown either by comparing animals of similar ages with and without partial removal or by comparing the growth of the transplants before and after total splenectomy in the same animal. The stimulus for this increased growth must be chemical in nature and must operate through the blood stream. The compensatory hyperplasia of the stump following partial removal of the gland cannot be separated from a possible nerve influence. The method of transplantation clearly demonstrates that specific nerves are not necessary for this reaction. Some investigators have not been able to obtain compensatory hyperplasia even of the stump *in situ* following partial removal, or of the thyroid, whose functions so far as we know cannot be assumed by any other tissue, and have doubted its occurrence, while others have no difficulty in demonstrating the effect even in transplants. In the work of Halsted¹⁰ and Hunnicut¹¹ on the thyroid their failure to obtain compensatory hyperplasia was probably due to their failure to induce a thyroid insufficiency, in the production of which two factors of the utmost importance are involved; *viz.*, the amount of thyroid removed and the presence of available iodine. Loeb¹² recently published his results on compensatory hypertrophy of the thyroid following partial removal in guinea pigs. He found that compensatory hypertrophy occurs, though it is necessary to remove nearly all the gland. We have found that in rats, rabbits, and guinea pigs it is necessary to

¹⁰ Halsted, W. S., Hypertrophy of the thyroid gland. Revision of experiments made 25 years ago, *Proc. Soc. Exp. Biol. and Med.*, 1912-13, x, 111.

¹¹ Hunnicut, J. A., The absence of hyperplasia of the remainder of the thyroid in dogs after piecemeal removal of this gland. Auto-transplantation of the thyroid in partially thyroidectomized animals, *Am. J. Med. Sc.*, 1914, cxlviii, 207.

¹² Loeb, Leo, Studies on compensatory hypertrophy of the thyroid gland. I, *J. Med. Research*, 1919, xl, 199.

remove relatively more thyroid in order to obtain compensatory hyperplasia of the remaining stump than in cats and dogs, and in all cases available iodine must be excluded because of its inhibitory effect.¹³ We believe that Loeb would have obtained more constant results had he in all instances allowed an interval of 30 days for compensatory hyperplasia to take place.

Histology of the Transplants.—This has been described elsewhere¹ and only brief mention of certain features need be made. Many of the transplants have reached 5 or 6 mm. in diameter (intracapsular measurements). They have all the general characteristics of normal spleen, both as to the number of component structures—capsule, trabeculae, lobules, Malpighian bodies, pulp, sinuses, blood pigment—and their relation to each other. No attempt has been made to demonstrate the presence or absence of smooth muscle fibers in the capsule and trabeculae. Apart from this the spleen is capable of complete regeneration. These studies indicate that while anatomically the spleen is very complex, biologically all the major elements are simple and endowed with uniform and marked regenerative capacity.

Permanence of Spleen Grafts.—Most of the experiments were terminated within a year. Two rabbits (Nos. 16 and 22) have been allowed to survive and at the examination on March 1, 1920 the transplants in each were found active and very vascular—1,181 and 1,062 days respectively. They are possibly slightly smaller than at the second examination 290 and 176 days after transplantation. Both rabbits were young at the time of transplantation and in both complete splenectomy was performed, thus insuring growth of the transplants. Both rabbits are still strong and active. One can conclude, therefore, that spleen autografts made under conditions which insure good initial growth are permanent. There appears, however, to be a slight involution or atrophy with age even in splenectomized rabbits, and, as already pointed out, transplants made in old rabbits without splenectomy may in time (several months) undergo complete atrophy. Our experiments with autotransplan-

¹³ Marine, D., and Lenhart, C. H., Colloid glands (goiters): their etiology and physiological significance, *Bull. Johns Hopkins Hosp.*, 1909, xx, 131.

tation of the spleen and also of the thyroid show that at least an anatomical deficiency is not necessary in order that transplants may take and remain active for several months. Growth of these grafts, however, usually does not occur unless there is a physiological insufficiency which may exist independent of the amount of functionally active organ. Loeb and Hesselberg¹⁴ have also shown that the taking of transplants is independent of a physiological insufficiency. Halsted,¹⁵ working with the parathyroid, concluded that it was necessary to induce a physiological insufficiency in order to obtain successful transplants.

Reaction of the Grafts in Acute Infections.—No experiments have been made relative to this point. In a few instances in which the rabbits died of pneumonia, the transplants were markedly congested, and in one rabbit (Experiment 19) which died of pneumonia, the transplants at autopsy were soft, engorged with blood, and microscopic examination showed increase in pulp cells. In the ordinary sporadic cases of pneumonia in rabbits the reaction of the spleen is so variable and even in healthy rabbits there are such variations that it would be necessary to carry out a series of experimentally controlled infections to obtain definite data.

SUMMARY.

No instance of survival of spleen homeografts beyond the usual taking and persistence for 1 or 2 weeks common to most homeografts has been observed, although the possible advantages of consanguinity, age, and splenectomy were fully utilized. This is in sharp contrast to thyroid, sex gland, and adrenal cortex homeografts, with which one may expect 10 per cent to survive the 30 day period. It suggests that spleen is a stronger antigen and excites a greater degree of immunity more quickly. With autografts survival and growth are the rule, and failures are due to technical errors. Age is an important factor in the growth of autografts. The younger the rabbit the

¹⁴Loeb, Leo, and Hesselberg, C., Studies on compensatory hypertrophy of the thyroid gland. II, *J. Med. Research*, 1919, xl, 265.

¹⁵Halsted, W. S., Auto- and isograft transplantation, in dogs, of the parathyroid glandules, *J. Exp. Med.*, 1909, xi, 175.

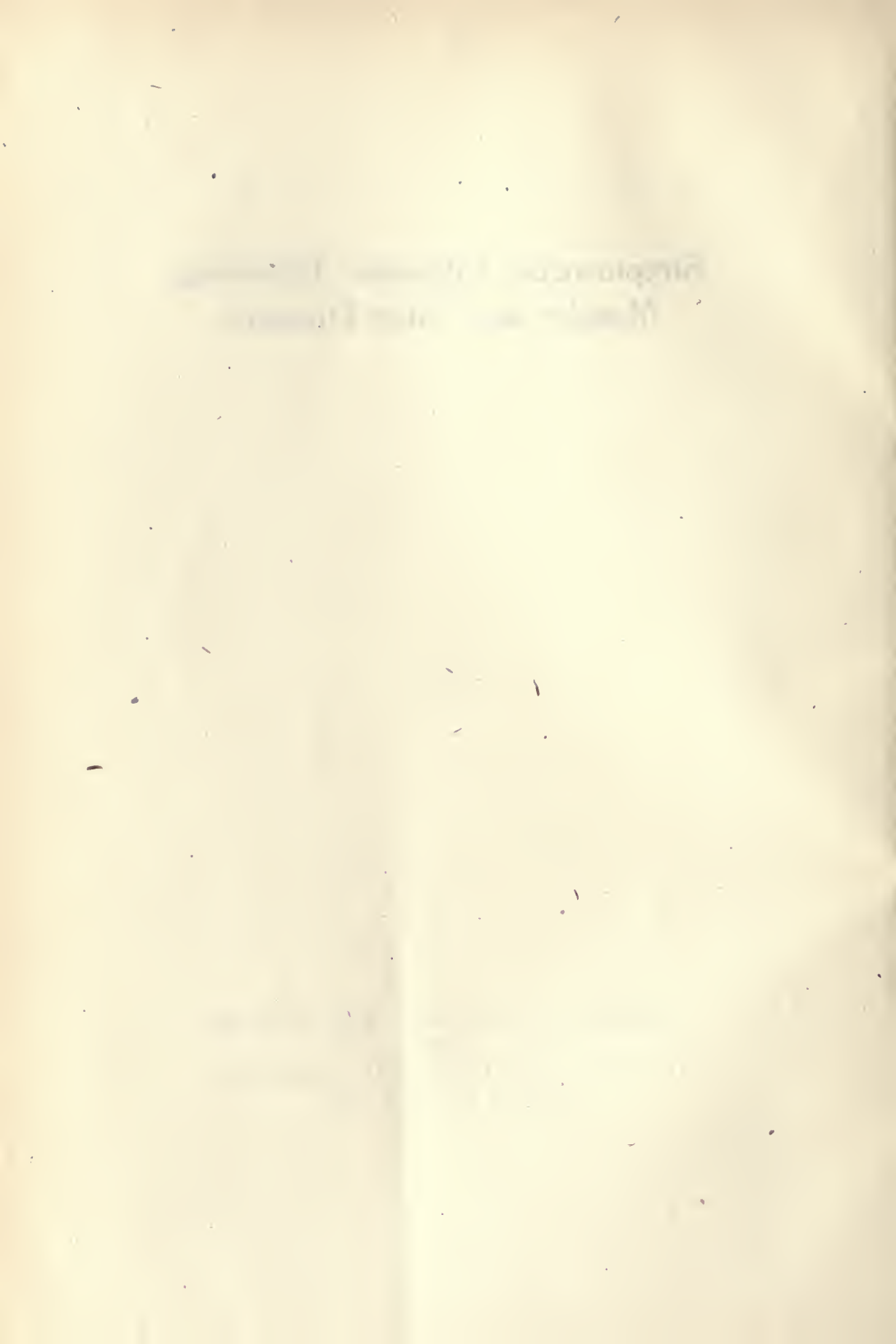
more growth is aided. This beneficial effect decreases gradually and becomes negligible after sexual maturity. Removal of the spleen is a powerful stimulus to the growth of transplants. The effect varies inversely with the age and usually is negligible after sexual maturity. The influence of age and splenectomy suggests that the spleen is most important in early life and after sexual maturity is either unimportant or its functions may readily be assumed by other tissues (hematopoietic). Anatomically the spleen is a highly complex structure, but biologically all the major elements of the spleen are simple as indicated by the uniform and marked regenerative capacity. There is a tendency for grafts to involute or atrophy with age, and grafts made in old rabbits without removal of the spleen may undergo complete atrophy. Grafts made in young rabbits, accompanied by splenectomy, have been observed for more than 3 years and may be said to be permanent. There is some evidence that subcutaneous autografts react to infections in the same way as the intact spleen.

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Streptococcal Infections Following Measles and Other Diseases

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STREPTOCOCCAL INFECTIONS FOLLOW- ING MEASLES AND OTHER DISEASES

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We have recently had opportunity to study the incidence and symptoms of streptococcal infections following measles, tonsillitis and other conditions associated with lowered resistance in soldiers, and to make observations on their pathology and symptomatology that are of assistance in diagnosis and in determining treatment.

During the fall and early winter up to the middle of December, 1917; approximately 200 cases of measles and fifty cases of rubella were treated, with complications in only three cases, otitis media in each instance, in one of which a mastoid infection required operation.

With the advent of "colds," acute bronchitis, pharyngitis and tonsillitis in the cantonment in late December and early January, a number of more serious complications appeared in measles patients as well as in soldiers in the hospital for other causes. The first one was a soldier convalescent from measles who was being held in quarters for the prescribed two weeks following his discharge from the hospital. The onset with chill, pain in the side, and fever suggested pneumonia, and pus was discovered by exploratory puncture only after several days of illness. Operation was attempted, but the patient died. Necropsy revealed that the entire process was not in any sense a true lobar pneumonia, but rather a lobular pneumonia followed by streptococcus pleurisy and empyema. Since the first case, all patients presenting pulmonary symptoms have been studied with the special object of

detecting empyema early and instituting drainage. Without the frequent and careful use of the exploring needle, a number of these cases would undoubtedly have passed for lobar pneumonia, or "pneumonia following measles." Indeed, we have been led to maintain a specially close watch on cases of supposedly frank lobar pneumonia, especially those showing organisms classed as Type IV, and have repeatedly detected empyema in such cases.

PATHOLOGY AND BACTERIOLOGY

The necropsy findings have been quite constant. Mathers has studied and reported a similar group of cases from civil practice. While the pleura is most frequently involved, other serous membranes, pericardium, endocardium and peritoneum, have also frequently been affected. In general the pathologic findings are those of septicemia with serofibrinopurulent pleurisy, pericarditis, mediastinitis, endocarditis or peritonitis, and in one case coincident arthritis. In early cases the inflammation is fibrinous, and there is much clinical evidence that the accumulation of fluid is a late and very rapidly developing process. In one case, occurring in the second week of measles, a primary peritonitis with initial symptoms referred to the right lower abdomen led to the drainage of a supposed atypical appendical abscess. At the necropsy an intact appendix, extensive pelvic, retrocecal and subphrenic peritonitis, recent general peritonitis, and secondary right empyema were found. In another case of general streptococcal infection with positive antemortem blood cultures following diphtheria, necropsy revealed terminal bronchopneumonia, acute purulent bronchitis, acute fibrinous pleurisy and left suppurative cervical lymphadenitis and gluteal abscess.

In the fourteen cases coming to necropsy, general serofibrinopurulent pleurisy, either primary or secondary, was present in all except one. The exudate was similar in all, lemon tinted serous fluid more or less turbid containing pus cells and enormous numbers of streptococci. Most of the pus cells were attached with fibrin to the pleura, forming a thick adherent matlike layer on the pleural surfaces. In some cases in addition to the main pleural involvement, smaller interlobar pockets of thicker yellowish green exudate

were found, some having the appearance of a somewhat older lesion in which granulations could be demonstrated. Bronchopneumonia, more or less extensive, sometimes almost lobar in distribution, was present in all. Compression atelectasis often made difficult the interpretation of the macroscopic examination. Mucopurulent bronchitis was present in all cases, and in many cases tonsillitis and laryngitis with edema were made out. As Mathers has pointed out, general lymphatic enlargement, peribronchial, mediastinal, cervical and mesenteric, is generally present. The spleen, however, was only slightly enlarged, and in some there was no appreciable enlargement.

It is perhaps a debatable question whether the course of events is bronchitis, bronchopneumonia and finally empyema, or whether the lesion is primarily a serous membrane infection with, in the pleurisies, secondary invasions of the lung. Certainly we have sometimes found such a rim of infiltrated lung lying next to the infected pleura. Possibly a middle ground may be taken, that in many cases the primary infection reaches the pleura by extension from a bronchopneumonia more or less extensive, but that in other cases the origin is hematogenous, as in the case of septicemia following diphtheria. Further evidence in favor of the direct hematogenous infection of serous membrane is found in the fact that in two instances the infection occurred first clinically in the peritoneum, pus being demonstrated by operation in one, and in both at necropsy; in one, peritonitis was evidently as old as, and in the other much older, than the accompanying empyema. In the latter a recent vegetative endocarditis was also present.

In all fourteen cases coming to necropsy, streptococci (usually hemolytic) were obtained by culture from the heart blood or spleen and from the pleural or pericardial or peritoneal exudates. In three cases, streptococci were recovered in blood cultures ante-mortem. In several cases also of the group of convalescent drained empyemas, streptococci were obtained in blood cultures during the height of the infection.

The sputums have been typed, some by the use of mice, some by direct culture in blood glucose broth. None of the organisms thus cultivated from these

cases has agglutinated with antipneumococcic serums of Types I, II or III.

During the latter part of the period of acute respiratory infections, large numbers of throat cultures of selected companies were being made for other purposes on blood agar plates. Hemolytic streptococci were observed in these cases in about 70 per cent. of the men examined, showing the widespread distribution of streptococcal infection in soldiers apparently healthy.

CLINICAL COURSE

In the majority of the thirty cases studied, the chief symptoms were pulmonary, with a history in most of these of "cold," and of severe cough in many. In several the history was that of a sudden onset with chill and pleural pain; but even in these a more careful inquiry elicited the statement that there had existed an antecedent bronchitis. In the case of primary peritoneal infection following measles, the first symptom was lower abdominal pain.

The physical signs are frequently misleading, especially in the cases in which the early course is that of pneumonia. In these, signs of consolidation, perhaps irregular in distribution, may be made out; and without marked alteration in physical signs, the more serious condition of the patient indicates an extension of the process. This extension may of course be due to additional lung involvement, or increase in severity of the septicemia; but not infrequently, exploratory puncture yields a streptococcus-laden pleural exudate. In other cases in which the physical signs have been carefully observed and recorded from day to day, the rapidity with which the empyema develops, with displacement of the apex beat and other coincident signs within a few hours has been repeatedly remarked. The puzzling physical signs have been in part explained in some of the cases in which operation has been performed, and in some at necropsy by the finding of extensive adhesions both old and recent, which prevented the distribution of pus, characteristic of fluid in a chest in which the pleura is free.

In some fatal cases in which pulmonary signs were of only four or five days' duration, the pathologic findings indicated that pleurisy, often interlobar, must have been present previously, at a time when the

patient was in relatively good physical condition with little or no fever.

In addition to frequent examination and the careful use of the exploring needle, the fluoroscope has been of assistance. Repeated leukocyte counts also have helped to determine an increase in the extent of the infection.

In the care of these cases it is important to bear in mind that this type of empyema is entirely different in its clinical course from the empyema that follows true lobar pneumonia. The exudate usually contains enormous numbers of streptococci, and on early operation and thorough drainage depends the chances of recovery. Even when early drainage is obtained, the infections of other portions of the pleura not accessible to drainage by reason of adhesions, the simultaneous infection of the pericardium, and the severity of the blood infection, preclude the possibility of recovery in some cases. In four of the fatal drained cases, vegetative endocarditis was found.

SUMMARY

Of the thirty cases, including fourteen fatal cases, twelve followed measles; and in eight, the physical signs of consolidation (in the cases examined at necropsy, bronchopneumonic in distribution), which preceded the demonstration of the empyema, led to a primary diagnosis of pneumonia. Our experience with these streptococcal infections suggests that while measles is an important predisposing factor, other infections, as bronchitis, tonsillitis, diphtheria, and other conditions, such as exposure and excessive fatigue, must be included—in short, anything that may reduce resistance to infection by an organism quite generally distributed during the epidemic of colds and bronchitis, in the noses and throats of the apparently healthy as well as the sick.

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A CONTRIBUTION TO THE TECHNIC OF ARTIFICIAL RESPIRATION IN MAN*

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THERE is a pretty general agreement that in attempting resuscitation in apparent death by electric shock, drowning, asphyxiation from gases, etc., artificial respiration should be at once begun by a manual method, the best being the prone pressure method recommended by Schäfer. The advantage, in many cases, of supplementing this later on, especially where the artificial respiration must be kept up a long time, by some mechanical arrangement neither liable to get out of order nor likely to be dangerous in the hands of a novice, has been pointed out by a number of writers whose acquaintance with the subject entitles them to speak with authority, and especially by Meltzer. Schäfer,¹ too, has remarked that the method of Horvath (forcing air into a nostril, by a bellows, the mouth being shut) "might well be employed in certain cases," although it "would not be available in most instances of apparent death from drowning; but its efficiency should not be forgotten, especially since it appears to afford a means of forcing air into the alveoli in cases in which the more gentle current of air which is produced by movements of the ribs, fails to find a passage through the frothy mucus which may partially block the bronchi."

Meltzer² quoting largely from Keith³ points out that inflation of the lungs by bellows was long the accepted method in resuscitation of the apparently drowned. It seems to have been discontinued on quite insufficient grounds. Anyone who has had experience of the routine methods of artificial respiration in animals in the laboratory, which can easily be carried on without any cutting operation by the use of a properly shaped mask or by laryngeal intubation,⁴ is aware of their great superiority over manual methods for long-continued respiration.

Meltzer has described a simple arrangement² which he considers superior to any of the more elaborate and expensive devices put upon the market by commercial firms. The current of air, maintained by a foot bellows, is interrupted at the desired rate by a respiratory valve actuated by a lateral movement of the thumb. A rubber bag is interposed between the bellows and the valve. Meltzer discards the close-fitting mask on the ground that the danger of infected material being forced into the lungs is increased by its use. For the mask he substitutes a pharyngeal tube of appropriate shape and size, the air being thus delivered to the patient by "pharyngeal insufflation." Others have raised objections to the "close-fitting" mask, and some have included in their condemnation all mechanical devices, the use of which according to them occasions neglect of the manual methods and delay in starting artificial respiration while the apparatus is being procured.

We have examined two forms of apparatus, Meltzer's and the commercial

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apparatus known as the "lung motor." Meltzer has shown that efficient respiration is easily maintained with his arrangement; a curarized dog, for example, being kept alive indefinitely. Although it seemed sufficiently obvious that this must also be the case with a pump like the "lung motor," we thought it worth while to demonstrate the point. There is no difficulty in maintaining with it efficient respiration indefinitely in a curarized dog. Of course, the mask must be of appropriate shape to fit a dog. One of the metal cones used for giving ether was found to answer admirably. A piece of sheet rubber was tied over the wide end and a round hole cut in the rubber, through which the dog's snout was pushed. The narrow end was closed by a cork, a tube in which communicated with the respiration apparatus. The animal's tongue was drawn out under the rubber and secured. This device is a convenient one for giving artificial respiration to animals which are to be allowed to survive, in an experiment or operation in which it is needed. It is theoretically an objection to the "lung motor" that the expiration is accomplished by a suction action of the pump. With a small animal the sounds accompanying this suction, extending widely over the thorax and even the abdomen, although apparently generated at the larynx, are alarming, but it was not possible to demonstrate that any harm was being done, so far as could be seen from the blood pressure curve or at autopsy. With a large animal the suction sounds were much less obtrusive. At the same time Meltzer's objection that this suction in expiration is unnatural and unnecessary seems to us a valid one, and it certainly seems better to allow the expiration to take place by the elastic recoil when the inflation ceases.

The foot bellows, with a rubber bag interposed, as recommended by Meltzer, appears to be the simplest and most practical method of obtaining the necessary pressure for inspiration. However, while there is no doubt that in skilled hands the pharyngeal tube is safe and efficient, we prefer a mask, as being simpler to adjust and better for general use. We believe that the mask for ordinary use should not be fastened in any way but merely held by the operator on the patient's face. This permits its removal at any moment for cleansing or for clearing out the mouth, and its immediate readjustment. We have also substituted for Meltzer's respiratory valve the valve shown in Figs. 1 to 3 (two-thirds of the actual size). The valve, constructed of aluminum, consists of a tube *T* (Fig. 1), one end of which is attached to the mask and the other end to a rubber bag kept filled with air by a bellows. A plunger *P*, is pressed in by the thumb or by the palm of the hand at the rate of fifteen to twenty times a minute. It is convenient to hold the valve with the four fingers applied to the tube, the plunger passing between the middle and ring fingers. When it is pressed in air passes through a hole bored across the plunger, and traversing the tube inflates the lungs. The position of the plunger in inspiration is shown in Fig. 2. The spring *S* (Fig. 1) is compressed as it goes in, and returns the plunger by its recoil as soon as the pressure ceases, allowing air to escape from the lungs through a hole bored longitudinally from the lower end of the plunger and then radially so as to open at one side of it, as shown in Fig. 3. The plunger carries a pin (*p*) which engages in the slot (*s*) and can move freely down the slot when the plunger is pushed in but can not return beyond the top of the slot when it is released. This prevents the possibility

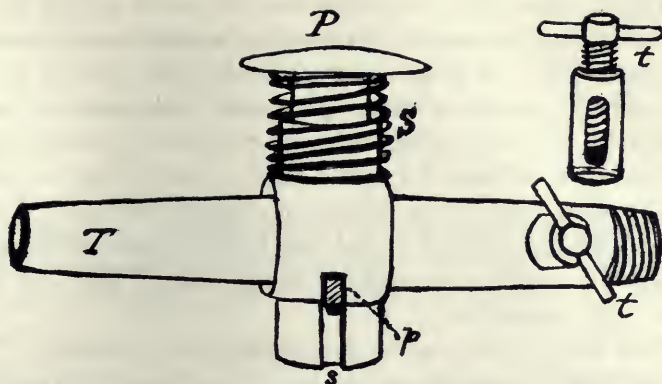


Fig. 1.—Respiration valve. *T*, tube through which air passes; *P*, plunger; *S*, spring; *p*, pin on plunger; *s*, slot in which *p* works; *t*, thumb-screw of safety opening shown separately in right-hand corner of figure.

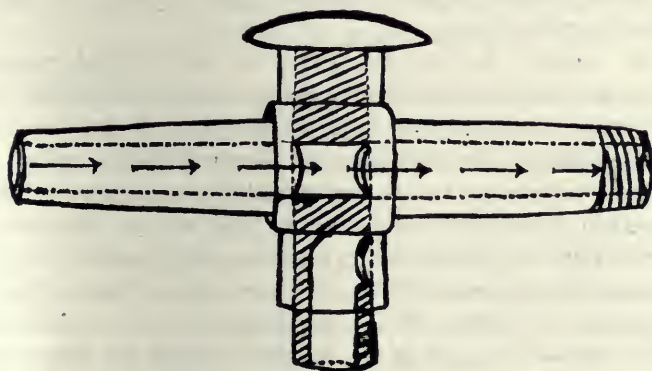


Fig. 2.—Section through respiratory valve showing position of plunger in inspiration.

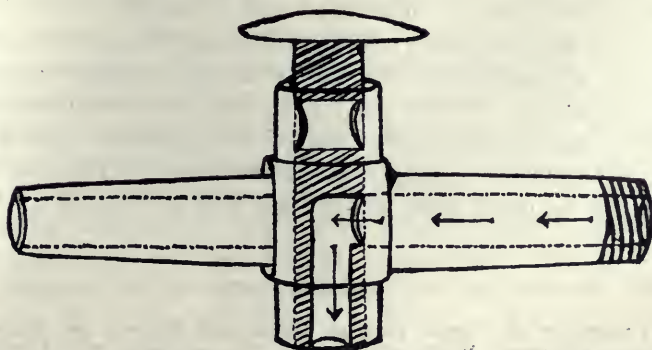


Fig. 3.—Section through respiratory valve showing position of plunger in expiration.

of the plunger coming out during the artificial respiration. A small thumb screw (*t*, Fig. 1) in a short side tube near the mask end of the tube *T* constitutes a safety arrangement to prevent the possibility of excessive pressure inside the mask. The side tube is slotted for the chief part of its length and by moving the screw, a greater or smaller permanent opening can be produced. The screw is arranged so that it is impossible to close the slot completely. The making of the safety arrangement and the valve in one piece we consider an improvement of some value, as it reduces the number of parts of the apparatus and, therefore, the time spent in connecting it up. A similar advantage is gained by the attachment of the valve directly to the mask. This also diminishes the dead space of the apparatus.

The valve can be taken apart in a moment for cleansing. To do this the plunger is removed by unscrewing the top. It is then easily pushed out of the tube *T*. The safety thumb screw (*t*) is also removable. The valve can be worked with great ease for long periods without fatigue. It can be operated by pressing with the first phalanx or the ball of the thumb or the palm of the hand. The valve is intended to be fastened to the mask so that one of the operator's hands suffices to work the valve and to hold the mask against the face, leaving the other free.

Since the operator may have to carry on the artificial respiration with the patient on the ground, an attachment has been fitted to the bellows which allows it to be worked conveniently by one forearm or one hand as well as by the foot. A wedge-shaped piece of wood is fastened to the top of the bellows so that its upper surface is nearly horizontal before compression. On this is hinged strongly a piece of wood about as long as the forearm. The hinge is situated at the edge of the bellows but the support projects two or three inches so as to increase the leverage. When the forearm is to be used, as is convenient when the operator is sitting down, the hinged piece is folded back over the bellows. The elbow is placed at the hinged end and the fingers project beyond and grasp the free end. Little effort is necessary in this way to keep the bellows going. When the hand is used, and it of course postpones fatigue in a long artificial respiration to vary the mode of compressing the bellows, the hinged piece is folded out so that it projects from the bellows and constitutes a lever.

As regards the question of what should be taught to the layman, our experience is in favor of teaching the immediate application of a manual method, followed by, or alternated with the employment of a simple mechanical device, when such is available. We do not believe that the average workman if taught to immediately begin with a manual method, the reason for this being sufficiently emphasized, and practiced in applying the mechanical device only later on when all is ready, will be at all likely to sit down and do nothing until an apparatus is procured.

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THE ELECTRICAL CONDUCTIVITY METHOD OF DETERMINING THE RELATIVE VOLUME OF CORPUSCLES AND PLASMA (OR SERUM) IN BLOOD

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Many years ago (1) I worked out a method of determining the percentage volume of plasma (or serum) in blood, based upon the fact that the corpuscles may be assumed to be non-conducting particles suspended in the plasma.

Although the method unquestionably gives more accurate results than the haematocrite and requires little time, it has not been greatly employed even in the laboratory, still less in the hospital. P. Fraenkel (2) recommended it for scientific purposes, after comparing it with Bleibtren's chemical method, and Wilson (3) compared it with the haematocrite method. However, he did not keep up the rotation of the haematocrite until the column of corpuscles had assumed a constant volume, but rotated for a fixed time (five minutes). This was done purposely because frequently the haematocrite is employed in this way. But although useful comparative results may be obtained on the same blood with a fixed time of rotation, this is not the case where bloods differing greatly in the percentage of corpuscles are to be compared.

As the measurement of the conductivity of physiological liquids is a much more familiar operation among biologists and clinicians than it was twenty years ago, and as we have been recently making use of the method again in calculating epinephrin concentrations in serum from the concentrations in the corresponding blood, and have had the opportunity to compare it rather extensively with the haematocrite method, I am confirmed in the belief that its advantages have not been sufficiently appreciated. To be sure, a larger quantity of blood is needed than that required to fill a haematocrite tube. I use a conductivity tube holding about 3 cc. and, therefore, must have 3 cc. of serum. But it is not difficult, as Wilson did, to employ a tube which requires only 4 to 5

drops of blood. The principle of the method permits the assumption that the formulae worked out on dog's blood will also be applicable to other bloods, since the corpuscles can always be taken as practically non-conducting.

In the previous paper the percentage volumes of serum as determined by the electrical method in thirty dogs taken at random are given. The range is from 39.6 to 74.0, and the average 55 per cent. The average for blood specimens taken from six of the dogs used in the recent work is 52 per cent of serum, the range 36.5 to 66.5. The results are compared with haematocrite readings in table 1.

TABLE 1

NUMBER OF ANIMAL	PERCENTAGE OF SERUM BY	
	Electrical method	Haematocrite
1	52.3	48 (5 minutes), 49 (10 minutes), 50 (15 minutes)
2	36.5	29.5 (40 minutes), 33 (60 minutes), 35 (70 minutes)
263	66.5	62 (15 minutes), 65 (23 minutes)
297	51.0	25.5 (10 minutes), 36 (20 minutes), 41 (30 minutes), 45 (40 minutes) 48 (50 minutes)
306	46.6	10 (10 minutes), 25 (20 minutes), 33 (32½ minutes)
307	62.0	

It will be observed that while great differences exist between the haematocrite readings with a given blood, according to the time of rotation, in no case does the percentage of serum with the longest time of rotation quite equal the percentage determined by the electrical method. The latter obviously constitutes a limit toward which the haematocrite readings approach more nearly the longer the rotation is continued. With some bloods the approach is extraordinarily slow (dogs 2 and 306, e.g.). Where the serum is scanty this is usually the case. In dog 306 after 32½ minutes rotation the end point had not been nearly reached. The percentage of serum as determined by the haematocrite at this time would have been less than two-thirds of the real percentage. The speed of the haematocrite was about 4000 turns a minute. A very high speed was purposely avoided so as to permit such differences to be readily detected. But with higher speeds they would still exist, although the end point would be reached sooner.

In table 2 is given a similar comparison on bloods from eighteen cats.

TABLE 2

NUMBER OF ANIMAL	PERCENTAGE OF SERUM BY	
	Electrical method	Haematocrite
1		73 (5 minutes), 73.5 (10 minutes), 74 (15 minutes), 74.5* (20 minutes)
2	62.5	57 (8 minutes), 60.5 (16 minutes), 62 (21 minutes)
8		71 (5 minutes), 71.5 (10 minutes), 72* (15 minutes)
9		51.5 (5 minutes), 53.5 (10 minutes), 54.5 (15 minutes), 55* (20 minutes)
91		87*†
214	71.0	
239	70.0	
259	76.5	65 (8 minutes), 70 (15 minutes), 73.5 (20 minutes)
284		50.5 (15 minutes), 51 (22 minutes), 52 (34 minutes)
285	56.5	46 (15 minutes), 49.5 (22 minutes), 52 (34 minutes)
286	67.3	32 (5 minutes), 57 (15 minutes), 60 (22 minutes), 62 (30 minutes)
287	44.7	30 (5 minutes), 40 (15 minutes), 41 (22 minutes), 42 (30 minutes)
288	67.1	
289	72.3	69 (15 minutes), 70 (25 minutes)
290	57.0	53 (10 minutes), 56 (20 minutes)
298	82.0	
305	53.8	43 (10 minutes), 48.5 (20 minutes), 51.5 (32½ minutes)
308		65 (10 minutes), 68 (15 minutes), 69.5 (20 minutes) 70* (25 minutes)

* With these bloods rotation was continued until the increment of the column of serum in successive rotation periods became negligibly small. It was seldom that an absolutely constant length was reached.

† This blood as it sedimented in the test tubes obviously consisted mainly of serum.

The average for the eighteen cats, including those where only haematocrite determinations were made, is 66.2 per cent of serum, the range 44.7 to 87 per cent. The average for all the cats used by us would certainly be distinctly higher, for a number of old animals with an abnormally low serum content for a cat are included in the table.

In accordance with the fact that the cat's corpuscles in general sediment much more rapidly on standing than the dog's, and also because of the lower proportion of corpuscles, the haematocrite readings approximate sooner to the percentage determined electrically. The latter still, however, constitutes a limit which is not exceeded by the haematocrite determinations. I have found the same to be true for human blood, for example, in a case of diabetes insipidus with anaemia, studied along with Christie (4). Wilson (3) invariably obtained lower serum

percentages by the haematocrite than by the electrical method in pathological cases. Probably the difference in these cases would have been reduced by longer centrifugalization. That the electrical method gives results which are approximately correct was established by comparison with two other methods in the former paper (1). It is strong corroborative evidence that the haematocrite readings come nearer and nearer to the values determined by the electrical method, the longer the rotation, without quite attaining them, since some serum is inevitably left in the sediment. In the electrical method no alteration whatever can be produced in the corpuscles, the measurement being made while they are normally suspended in the serum.

Since the viscosity of blood is influenced greatly by its content of corpuscles, it is easy to see that very considerable differences may exist in the coefficient of viscosity of the blood of healthy individuals of the same species and in the average viscosity of the blood of different kinds of animals. Burton-Opitz has shown this in his elaborate investigations on the viscosity of blood. He found that the viscosity of dog's blood was on the average five times greater than that of distilled water (at 37°C.), that of rabbit's blood only 3.4 times greater, while cat's blood possessed an intermediate value.

Lewy (6) also observed a great difference in the viscosity of blood from different animals of the same species as well as in animals of different species. Welsh (7) found a considerable range in healthy human beings and of course very great variations in disease.

The fact that great variations in the viscosity are compatible with a perfectly efficient circulation, suggests that the emphasis which has been placed on the superiority of such substances as gum or gelatine as constituents of transfusion liquids is scarcely warranted. In so far as they may attract water from the tissues and retain it in the circulation, they may have some advantage. But when it is argued that since gum or gelatine solutions by increasing or maintaining the viscosity of the blood enable a greater arterial pressure to be attained, they must be superior to simple salt solutions, the physiological basis for the conclusion seems open to question.

Why should it be necessarily advantageous to artificially increase the resistance which the heart must overcome in order to drive blood through the tissues? When the viscosity is increased the resultant rise of blood pressure, provided the response of the heart is adequate, does not mean as a matter of course that the tissues are getting more blood than before but may merely mean that the heart by working harder

is able to deliver to them as much blood as before. It is true that the physiologist or the physician who is estimating the blood pressure may have the satisfaction of seeing it mount toward what is considered a normal level, but beyond this it is not clear that there would be any necessary advantage. If the response of the heart is inadequate and the rise of blood pressure is insufficient to overcome the extra resistance, the blood flow in the tissues may be diminished although the pressure has been increased. Of course, it may be argued that a certain minimum blood pressure is essential and that if this were not reached the organs, including the heart, would not function properly, even if a sufficient blood flow through them could be maintained. This, however, is one of the moot points of physiology, while there is general agreement that the important thing is a sufficient flow of blood. There are certainly conditions in which this essential blood flow might be promoted by diminishing the viscosity of the blood. If no other change occurred the blood pressure might be expected to fall. But where the blood viscosity is diminished by the injection of salt solutions the volume of the circulating liquid is at the same time increased, and if the pressure does not fall (it is not necessary that it should rise) the blood flow will be increased. When blood corpuscles are injected the viscosity is necessarily increased and the flow to that extent rendered more difficult. But there is the compensating advantage, which is most obvious in haemorrhage, that the increased viscosity is due to the addition of essential elements to the blood, an advantage which, of course, does not exist in the case of gum. These remarks are not intended in any way to prejudge the question whether clinical experience or physiological experiments may not have demonstrated the superiority of solutions containing such substances as gum.

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ON THE LIBERATION OF
EPINEPHRIN FROM THE
ADRENAL GLANDS

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ON THE LIBERATION OF EPINEPHRIN FROM THE ADRENAL GLANDS*

WITH DISCUSSION OF SOME OF THE METHODS EMPLOYED IN ITS INVESTIGATION

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THE organs which furnish internal secretions are attracting the interest of many physiologists, and among this group of important glands the suprarenal bodies have been the subject of considerable investigation, which has resulted in the accumulation of voluminous literature. From time to time various attractive theories have been formulated, some based upon experimental evidence and others being chiefly speculative, in attempts to solve the problems concerning the functions of these glands. The principal result achieved through these theories is the stimulation of the interest of numerous investigators, resulting in the accumulation of data tending to support one or another of the theories, or, on the other hand, establishing evidence which contradicts the fundamental principles upon which they are based. Some of the prominent theories that have been proposed are as follows: that the adrenal glands neutralize toxins circulating in the blood; that the internal secretion of the glands maintains the normal vascular tone; that the glands are interrelated with other ductless glands, directly and indirectly influencing metabolism; that the adrenals have an emergency function, liberating outbursts of epinephrin in times of special stress in response to emotions such as fear, anger, rage, etc., resulting in the mobilization of the defensive forces through the influence of the liberated epinephrin in the blood and its action on the sympathetic nervous system. However, careful consideration of the literature leads only to the conclusion that we are still far from the solution of the problem concerning the function of the adrenals.

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Out of the mass of experimental work on record, there are but few facts that can be accepted as having been definitely established, and these facts at present lend little, or no support, to any of the existing views of the functions of these glands. With the rapid advance being made in the development of physiologic and biochemical methods of investigation it may be hoped that substantial information will be obtained in due time.

Our knowledge of the function of the adrenal glands begins with the observations made by Addison¹ in 1855, that in the disease, which now bears his name, the adrenal glands are the principal seat of pathologic changes. This was followed by experimental investigations made by Brown-Séquard² in 1856, who found that these glands are of vital importance, their complete removal in animals resulting in death. The nature of the material manufactured by the adrenals was investigated by Oliver and Schäfer³ in 1894, and by Szymonowicz⁴ in 1895, by injecting into the circulation of animals, extracts of suprarenal glands. They observed that this caused a marked increase in the blood pressure. Later investigations concerning the adrenals were greatly facilitated by the isolation of an active constituent of the glands, which possesses the same blood pressure raising quality as extracts of the gland. In 1897 Abel⁵ obtained an active product from the adrenals (epinephrin), and in 1901 Aldrich⁶ and Takamine,⁷ independently, isolated an active product (adrenalin). This active principle was prepared synthetically by von Fürth⁸ in 1898 (suprarenin), and by Dakin⁹ in 1905.

The observations of Lewandowsky¹⁰ and of Langley¹¹ indicated that the active product of the adrenals produced the same effect upon certain structures which receive nerve supply from the sympathetic system, as is caused by electric excitation of their sympathetic innervation. An extensive study of the actions of adrenalin on various structures or organs was made by Elliott¹² and he showed clearly the sympathomimetic action of this substance. This action of adrenalin led to the use of certain structures, which are innervated through the sympathetic system, as biologic test objects whereby the active product of the adrenal glands can be detected and estimated.

A very delicate means of detecting epinephrin in blood is afforded by the use of segments of rabbit's intestine and uterus. Cannon and de la Paz¹³ employed for this purpose strips of longitudinal muscle of cat's intestine. Stewart¹⁴ and Hoskins¹⁵ utilized segments of rabbit's intestine and Stewart showed that it is advantageous to use, as an additional test, segments of the rabbit's uterus. The intestine segment, contracting rhythmically in ordinary (venous or arterial) blood, when brought in contact with epinephrin-containing blood responds by inhibition of the tone and contractions of the segment, while the uterus segment indicates the presence of epinephrin by the opposite effect; i. e., by an increase in tone. The importance of corroborating a reaction produced by a specimen of blood upon the intestine with that shown by the uterus, or some other object which reacts in a different manner than the intestine, is evident, since a number of substances other than epinephrin are capable of causing inhibition of the intestine. In fact, minute changes in the concentration of the salts in the fluid which surrounds the segment or alteration of the oxygen supply may cause inhibition. When the inhibition of the segment is due to epinephrin, it can be con-

firmed by the increase in tone produced when the same specimen of blood is applied to the uterus.

Since the proper interpretation of results obtained with the intestine and uterus segments depends greatly upon the manner in which the test is applied, it is not out of place to describe briefly the method of testing blood upon these objects. The apparatus in which the blood is tested consists of a cylinder, in the bottom of which is a hook for the attachment of the segment. A capillary tube enters the side of the cylinder near the bottom. Through this a constant supply of oxygen is kept bubbling through the liquid which surrounds the test-object; the cylinder is kept in a water-bath at a constant temperature of about 38° C. The segment is attached at one end to the hook in the cylinder and at the other end, by a thread, to a lever which records the contractions. The fluid which surrounds the segment is introduced with the aid of a narrow pipette, properly bent and its end drawn out so that it can enter between the segment and the wall of the cylinder, thus permitting the fluid to be introduced at the bottom of the cylinder, so that, as it enters, it can displace the fluid in which the segment is beating. When the test for epinephrin is applied, the segment, contracting rhythmically in Ringer's fluid, is surrounded by ordinary (usually venous) blood which displaces Ringer's solution as it enters the cylinder. The blood to be tested is then introduced through the pipette at the bottom of the cylinder and as it enters it displaces the indifferent blood and the presence of epinephrin is indicated by an inhibition of the tone and contractions of the intestine segment. A doubtful reaction can always be rendered more certain by applying the test to a segment of the rabbit's uterus. When very small quantities of epinephrin are present in a specimen of blood, the test is facilitated by employing the serum, in which the concentration of epinephrin is greater, instead of the whole blood, for it has been shown that in a blood which contains epinephrin, the serum contains the total amount present in the blood.¹⁶ Some investigators remove one fluid from the cylinder before introducing another. This may be a source of error, as exposure of the segment to the air may alter its sensitiveness in succeeding tests. Also, on removal of one liquid, and then replacing another (or this same liquid), the segment will sink, then be floated up with the emptying and refilling of the cylinder, respectively. This will cause the writing lever to record a rise when the segment sinks, and then a fall as the segment is floated up, which may easily be incorrectly interpreted as an inhibition. Displacing one fluid in the cylinder with another by introducing through the pipette with its orifice at the bottom of the cylinder avoids these possible sources of error. The possibility of incomplete displacement by admixture of the fluids is minimized or completely avoided by introducing more displacing fluid than is necessary to just fill the cylinder. Quantitative experiments made in the course of our work with this method have shown that a given specimen of blood yields constant results when applied in successive observations to the same segment. A large number of observations can usually be made on one segment, but it is important, to be certain, when comparing the effects produced by various specimens of blood, that the condition of the segment has not altered. A reaction obtained by one specimen early in the series of observations can not be compared with one obtained by a different specimen later on, unless it can be shown by repeated

applications of different specimens of blood that the sensitiveness of the test object is the same.

Since extracts of the adrenal glands, when introduced into the circulation, are capable of influencing the blood pressure, it is desirable to know whether a substance possessing this activity is given off by the glands to the blood which passes through it. To determine this two means are available: (1) the withdrawal of blood through a cannula from the adrenal veins, and testing it on the biologic test objects, or observing its effect when injected into the circulation of another animal; (2) observations in which the liberation of epinephrin is deduced from changes in the blood pressure or other reactions in one and the same animal, without withdrawing the adrenal vein blood.

Evidence of liberation of epinephrin from the adrenals was first obtained by stimulation of the splanchnic nerves. Dreyer¹⁷ and Tscheboksaroff¹⁸ found that blood collected from the adrenal veins of dogs during stimulation of the splanchnic nerves produces a distinct rise in blood pressure when, after defibrination, it is injected into the circulation of another dog.

Employing rabbits' intestine and uterus segments, it was shown by Stewart¹⁹ that blood collected from the adrenal veins during splanchnic stimulation or during massage of the gland, indicated the presence of epinephrin.

Withdrawal of blood, and its defibrination subject it to possible alteration; therefore, it is desirable to correlate the observations already mentioned with results of experiments made on one and the same animal without withdrawing the adrenal vein blood. Meltzer²⁰ has shown that when the superior cervical ganglion has been excised in a cat, the pupil on the deganglionated side (which becomes constricted) within a few days becomes especially sensitive to adrenalin, dilating widely when adrenalin is introduced into the circulation, while the normal eye remains unchanged. By means of this reaction, Joseph and Meltzer²¹ found that stimulation of the peripheral end of the splanchnic nerve in an animal so prepared caused dilatation of the deganglionated pupil. They explained this on the hypothesis of the liberation of epinephrin from the adrenals into the circulation. Stimulation of the splanchnic in the animal in which the reaction is to be elicited renders it necessary to exclude the possibility that the reaction is a nervous phenomenon due directly to the stimulation of the nerves. Asher²² attempted to determine this point by excising the abdominal viscera, leaving the adrenals intact, then stimulating the splanchnic nerve with the adrenal veins alternately clamped and open. He studied the effects upon the blood pressure and observed that a rise was obtained when the adrenal veins were open, but when they were clamped no rise in blood pressure was caused by splanchnic stimulation. Employing Meltzer's reaction, previously described, Elliott²³ confirmed Meltzer's observation and also found that in cats this reaction is elicited by splanchnic stimulation when the adrenals are intact but could not be obtained after excision of the glands. He further observed that when one adrenal is excised, stimulation of the splanchnic on that side is without effect, while stimulation of the nerve on the side in which the adrenal is intact causes the characteristic effect upon the pupil.

Further evidence that this reaction is obtained through the liberation of epinephrin into the blood coming from the adrenal glands when the splanchnic

nerves are stimulated or the glands massaged has been found in a number of ways by Stewart, Rogoff and Gibson.²⁴ It was shown that when blood from the adrenals is prevented from reaching the eyeball, as by clamping the adrenal veins or the vena cava (above the orifices of the adrenal veins) during splanchnic stimulation or massage, no reaction is evoked; but when the clamp is released, the reaction occurs after a time interval corresponding to the circulation time necessary for blood to travel from the adrenal to the eyeball. The time required for this reaction to occur can be varied by variations in the rate of the circulation; i. e., slowing the circulation by vagus stimulation or by hemorrhage causes an increase in the time necessary for the reaction to occur, the difference corresponding to the difference in the rate of the circulation. The reaction evoked by massage or splanchnic stimulation under all of the conditions mentioned can be imitated by injecting the proper amount of adrenalin into the circulation at the level of the adrenals, the time necessary before the reaction occurs corresponding with the circulation time as modified by the conditions interposed. The latent period for the secretion of epinephrin is apparently very short, since the time interval necessary to obtain a given reaction is the same with splanchnic stimulation as when a corresponding amount of adrenalin is injected into the circulation at the level of the adrenals.

The evidence of liberation of epinephrin into the circulation from the adrenal glands in response to artificial stimulation of their splanchnic innervation is conclusive, and the next question to consider is whether it is possible to demonstrate liberation of epinephrin from the glands in the absence of artificial stimulation of their nerves, or what may be termed a "spontaneous liberation."

It was found by Tschoboksaroff¹⁸ that adrenal vein blood collected in the absence of splanchnic stimulation caused a smaller rise in blood pressure, when intravenously injected into another dog, than blood collected during stimulation of the nerves, and that after section of the splanchnic nerves the result indicated a distinct diminution in the adrenalin secretion. O'Connor²⁵ compared on the frog perfusion preparation (Laewen), the constrictor effects of rabbit's adrenal blood collected before and after section of the splanchnics, and observed a greater vasoconstrictor effect to be produced by the blood obtained before the nerve section. He also showed that shed blood develops vasoconstrictor substances. Trendelenburg²⁶ further observed that these substances are developed in citrate plasma so rapidly (a fraction of a minute) that the perfusion test for epinephrin when present in small quantities can not be entirely reliable.

It is desirable, therefore, to employ a method which eliminates the obvious difficulties entailed in using shed blood and vasoconstrictor reactions for the tests. A convenient method was employed by us,²⁷ working with cats and dogs. The blood coming from the adrenal veins was collected in a pocket of the vena cava and then released into the circulation, and simultaneous observations made upon the effects produced on the blood pressure of the animal and the eye reactions of Meltzer. The cava pocket is made by tying off the lumbar and renal veins, and all small branches which enter the cava from the liver to the bifurcation of the iliacs; thus a clamp adjusted just below the liver and one just above the iliacs completes a blind pouch into which only the adrenal veins are emptied. Blood collected in such a pocket is released by removing the upper

clamp and the reactions obtained represent those of unaltered adrenal vein blood. By timing the filling of the pocket and also determining the capacity of the pocket, it is possible with this method to determine the rate of blood flow through the adrenals, and by imitating the reaction obtained when the pocket is released by the injection of proper amounts of adrenalin intravenously, it is also possible to estimate the rate of liberation of epinephrin from the adrenals. It has been shown by this method, with the blood pressure and eye reactions, that in the absence of splanchnic stimulation; i. e., spontaneously, a liberation of epinephrin takes place, varying within narrow limits from 0.0008 to 0.0028 mg. per minute in the cat (or from 0.0003 to 0.001 mg. per kilogram of animal). After section of both sympathetics in the thorax near the diaphragm, including both splanchnics, the spontaneous liberation was no longer detected by these reactions, but section of the splanchnics alone in the abdomen did not always entirely abolish the liberation of epinephrin.*

Electrical stimulation of the peripheral end of the cut nerves during collection of blood in the pocket again elicited the reactions previously obtained. The same result was obtained when the pocket blood was tested upon rabbit's intestine and uterus segments.²⁸ As the adrenal vein blood, when the segment test is applied, is not diluted with the general blood, as is the case with the test described, these test objects are capable of detecting smaller amounts of epinephrin in the pocket blood than could be detected by the blood pressure or eye reactions. The question may be raised whether this is a normal liberation, or is due to the experimental conditions (trauma, anesthesia, etc.). Although some authors seem to assume that trauma and other experimental conditions will necessarily increase the spontaneous liberation, no experimental proof of this has ever been given. The fact that the output, as experimentally determined, varies within rather narrow limits in different cats, notwithstanding differences in the degree of trauma and the nature and depth of anesthesia, is much in favor of the view that the liberation is a normal phenomenon. We have shown that the anesthetic has nothing to do with it; for (a) the output of epinephrin is within the ordinary limits when anesthesia is produced without the use of a chemical anesthetic, as by a pressure bag inserted intracranially; (b) in animals whose spinal cord has been severed, in the cervical region, some days before the experiment and in which, accordingly, no anesthetic was necessary for testing the adrenal vein blood, the output was also within the usual limits.

It has been established beyond doubt that the adrenal glands continuously secrete a certain normal amount of epinephrin, and that electric excitation of their nerves causes liberation of epinephrin to take place. This leads to the question whether certain physiologic processes are capable of influencing the quantity of epinephrin liberated from these glands.

It has been stated by some writers that the adrenal glands are stimulated to increased secretion of epinephrin by such influences as asphyxia, stimulation of sensory nerves,²⁹ emotional disturbances¹³ and by the introduction into the

*It is known that all of the secretory fibers to the adrenals do not come from the splanchnic nerves, some of them being given off from the sympathetic lower than the origin of the splanchnics. If there is still a detectable liberation of epinephrin, after section of nerves to the adrenals, some of the nerves going to the glands must have escaped section. In the entire absence of innervation, the adrenals do not liberate epinephrin, and do not seem to regain this power, while the formation is apparently not interfered with after section of the nerves. It must be assumed that after the store, in the glands, has been replenished, the formation stops.

circulation of certain gland extracts.³⁰ The experimental evidence offered as proof of such an effect upon the adrenal glands is not substantial, as it is based upon investigations made with methods which can not yield reliable results.

For these investigations, blood was collected, with the aid of an aspirator, from the inferior vena cava at the level of the adrenals, by inserting a flexible catheter through an incision in the femoral vein (in cats) so that the opening in the end of the catheter was just anterior to the orifices of the adrenal veins. Specimens of blood were obtained through the catheter before and after causing asphyxia, etc., and were tested for epinephrin on intestine strips or segments. Inhibition of the test object caused by applying the specimen of blood collected after the experimental condition was induced was interpreted as indicating an increase in the amount of epinephrin liberated from the adrenals. It must be pointed out that only the intestine was used as a test object and, as previously stated, this test can be considered reliable only when it is possible to confirm the reaction by some other test object. Indeed, Cannon and Hoskins²⁹ state that ordinary venous blood collected from an animal during asphyxia causes inhibition of the intestine segment, although they show that this is not due to epinephrin. This fact has been repeatedly observed in the course of our work in this laboratory, and with the aid of the uterus segment we have always been able to eliminate the possibility of such a reaction being due to epinephrin, as it also caused inhibition of the tone of the uterus, while epinephrin has the opposite effect on this test object.

If the blood collected through a catheter from the adrenal level causes an inhibition of the intestine which is due to the presence of epinephrin, this yields no information on the rate of liberation of epinephrin from the adrenals. For this, it is essential to know the rate of the blood flow. A high concentration of epinephrin in the blood may be accompanied by a correspondingly slow blood flow through the adrenal or through the cava, while the liberation of epinephrin from the gland remains constant. It has been shown³¹ that within a wide range of variation of blood flow through the adrenals the liberation is approximately constant, and that the concentration of epinephrin in the adrenal vein blood varies inversely with the rate of blood flow through the glands.

It is conceivable that changes in the circulation induced during asphyxia, etc., are capable of sufficiently altering the rate of the circulation in the inferior cava to alter the concentration of epinephrin in the blood which is collected from the cava near the adrenals, but this is not an indication that any change in the rate of liberation of epinephrin from the adrenals has taken place.

Although we have recognized that the catheter method can not yield substantial information, we have made experiments, with this method, on the influence of emotions, asphyxia, and afferent stimulation on the liberation of epinephrin, and have been unable to confirm the results that have been reported.

We have studied the influence of asphyxia and sensory stimulation (of large nerve trunks) upon the liberation of epinephrin from the adrenals,²⁷ employing the "cava pocket" previously described, with the blood pressure and eye reactions. No effect upon the rate of liberation could be detected by this method. The same result was obtained with the adrenal vein blood, obtained from the cava pocket when tested upon rabbit's intestine and uterus segments.^{32, 33} These

observations permitted the study of the rate of blood flow through the adrenals, as well as the concentration of the epinephrin in the adrenal vein blood, and any increase in the concentration could always be accounted for by a corresponding diminution in the rate of blood flow, indicating that no detectable change in the rate of liberation from the glands had taken place during asphyxia or stimulation of the sensory nerves.

Cannon³⁴ has reported, in a preliminary communication, that he has devised a new method whereby he believes an increased activity of the adrenal is evidenced during asphyxia by a rise in blood pressure which he fails to obtain when the adrenals are excised. The recent observations of Gley and Quinquaud,³⁵ however, indicate that the rise in blood pressure during asphyxia does not depend upon secretion of epinephrin from the adrenals, for they find no diminution in the rise with asphyxia after ligation of the suprarenal veins. This is against the idea that asphyxia causes any sensible increase in the rate of liberation of epinephrin.

The influence of certain drugs in diminishing the content of epinephrin in the adrenals has led Elliott²³ to interpret the diminished store as due to an increase in the liberation of epinephrin from the gland. He concludes that since morphine, in the cat, produces effects which simulate the general appearance of fright, the resulting diminution of epinephrin store in the adrenals must be due to the increased liberation of epinephrin from the glands in consequence of the emotional excitation or "morphine fright," although he did not directly study the influence of frightening the animals. The effect of morphine in depleting the store of epinephrin from the adrenals has been confirmed by us.³⁶ But it has been shown that this effect has nothing to do with the emotional state of the cat, since in the dog and rabbit morphine also depletes the epinephrin store from the gland, although the physiologic picture of the animals is the opposite of that in the cat, the narcotic action being manifest. It must be emphasized that diminution of the store of epinephrin in the gland can not be assumed without proof to be the result of increased liberation, as the epinephrin content of the adrenals at any time can only represent the balance between production and liberation. If the rate of production is lessened, the store may be diminished without any change in the output. And contrariwise, if the production is increased, the rate of liberation may also be increased without any diminution in the store. For example, a considerable amount can be caused to be liberated by electric excitation of the splanchnics without noticeable influence on the store of epinephrin in the gland.^{24, 36}

There is at present no obvious method by which the influence of emotions upon the rate of liberation of epinephrin from the adrenals can be properly studied. It is not permissible to assume that the symptoms of sympathetic excitation which are seen in a frightened animal must be due to the action of epinephrin liberated in large amounts from the adrenal glands, for the same symptoms can be elicited in animals so prepared that very little or no epinephrin is being given off from the adrenals. By excision of one adrenal and denervation of the opposite gland in the cat it has been shown that the animal can live indefinitely in apparently excellent health, although the operation has reduced the epinephrin from the adrenals to a very small fraction of the normal, and in

many cases to quantities too small for detection with extremely sensitive test objects, indicating practically no liberation whatever.²⁸ In some of the cats it was found that the liberation of epinephrin from the adrenals was so interfered with by this operation that there could not have been one-thousandth of the normal liberation, if any epinephrin was being given off from the glands. These animals, nevertheless, responded readily to fright and other emotional disturbances with the usual symptoms of sympathetic excitation, in the same manner as normal cats. Certainly, an outburst, through nervous influence, of epinephrin in such quantities as would be necessary to produce these symptoms would be impossible in these animals.

The conception of an emergency function of the adrenal gland lacks substantial evidence. It involves essentially the occurrence of sudden outbursts of epinephrin far above the normal amounts liberated. It has not been shown that the liberation of such amounts of epinephrin can be induced. Even if it could be proved that during such emergency conditions the adrenals responded with augmented liberation, the suggestion that this has a bearing upon the emergency mobilization of sugar in the body, as evidenced by hyperglycemia³⁷ is not tenable, since it has been shown that cats so prepared that the liberation of epinephrin from the adrenals is reduced to quantities too minute for detection are still capable of responding to the usual procedures (asphyxia, etherization, etc.) employed in producing experimental hyperglycemia.³⁸ The normal blood sugar in these animals was the same as that found in unoperated animals. This is against the idea of the existence of a hypoglycemia due to adrenal deficiency (said to exist after adrenalectomy, in which the animal is moribund). Further evidence against the view that these hyperglycemias are associated with an increased liberation of epinephrin from the adrenals, or that they are similar to the hyperglycemia produced by injection of adrenalin, is the well-known fact that subcutaneous injection of adrenalin causes hyperglycemia more readily than intravenous introduction.

Animals, with practically no epinephrin being given off from their adrenals, as previously stated, are capable of responding to fright, rage, fear, etc., in the same manner as normal animals. They seem to be as capable of muscular exertion and as combative as the normal animals. The fact that such cats live indefinitely in apparently good nutrient condition suggests that epinephrin is probably not indispensable to life and health, unless the retroperitoneal and other chromaffin tissue in the body, which has been shown to contain epinephrin,³⁹ can compensate for the loss of epinephrin secretion from the adrenals. It has not been shown, however, that such chromaffin tissue liberates epinephrin. It is possible that some other substance, at present unknown, the liberation of which is under nervous influence, may be found in the secretion of the adrenals, the lack of which is responsible for loss of life when the adrenals are extirpated.

A number of writers have attempted to show that the secretion of epinephrin from the adrenals is influenced by shock. Some of the conclusions offered are based upon histologic appearance of the glands, indicating a loss of chromaffin substance. This can not be taken as evidence of an increase in the liberation of epinephrin, for the same reason discussed previously in relation to conclusions based upon a decrease of epinephrin store in the glands; nor is it likely that his-

tologic examination of the glands could yield definite quantitative information.

An investigation in which adrenal vein blood was collected before and after induction of shock and the bloods tested upon intestine segments was reported by Bedford.⁴⁰ He concludes that the rate of liberation of epinephrin from the adrenals in dogs is augmented by shock produced in several ways. The tracings accompanying the paper do not indicate in what manner the test objects were subjected to the action of the bloods. No satisfactory estimations were made to determine quantitatively the amount of epinephrin in the adrenal vein blood collected before and after shock was induced. On careful consideration of the results reported, the only definite information that can be gathered is that if the inhibitions of the intestine were due to epinephrin in the bloods tested, the specimens collected after the induction of shock had a higher concentration of epinephrin than the specimen collected before shock was induced. This increased concentration seems to correspond closely with a fall in the blood pressure. As already pointed out, it is to be expected that in a condition of low blood pressure, when the rate of blood flow through the adrenals is markedly slowed, there will be a relative increase in the concentration of epinephrin in the adrenal blood, although the rate of liberation may be unaltered.

In view of the fact that the adrenal glands secrete a substance so potent as epinephrin, and since it has been shown that the liberation of this secretion into the circulation is sustained through the influence of nerves which are in connection with a definite center in the spinal cord,⁴¹ it would seem not unlikely that certain experimental procedures, such as asphyxia, stimulation of sensory nerves, etc., might be capable of influencing the liberation of epinephrin, reflexly, through this center. This must not be assumed as a fact, however, until it is proved.

Premature attempts on the part of clinical men to apply what is at present known of the physiology of the adrenals, at least when risk to the patient is involved, are to be deprecated. Certainly, the present state of our knowledge of the subject does not warrant its clinical application to the extent of attempting adrenalectomy as a measure of relief in conditions of arterial hypertonus associated with Bright's disease, on the assumption that this condition is associated with hyperadrenalinemia, as has been done.

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THE OUTPUT OF EPINEPHRIN IN SHOCK¹

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In most of the experiments the classical method of producing experimental shock, exposure with or without manipulation of the intestines, was adopted. "Manipulation" in our experiments included displacement, handling, pinching firmly between the fingers and thumb, flicking with the fingers, tapping with such objects as the handle of a scalpel, etc. Compression of the inferior vena cava till a condition of permanently low blood pressure was induced was also employed, since this method has been extensively used of late in the study of shock. We do not, however, consider this the most suitable method when the behavior of the adrenal is to be studied because of the possibility that a more or less prolonged period of passive congestion of the gland may be a disturbing factor. In one or two experiments a state of continued low blood pressure was induced by hemorrhage and in one experiment by the injection of "peptone." It is not to be imagined that exactly the same physiological changes are brought about when the blood pressure is permanently lowered in all these different ways. On the contrary, the condition produced by peptone injection obviously differs in important features from the condition which follows copious hemorrhage and this from the condition or conditions produced by the exposure of the intestines or partial occlusion of the cava, which are perhaps more properly termed experimental shock.

It was in order to increase the chance of finding a procedure which would grossly alter the rate of output of epinephrin that so many and

¹ About six months ago I was asked to become a member of the Sub-Committee on Shock (Medical Division of the National Research Council), the work more particularly assigned being an investigation of the output of epinephrin in that condition. The investigation has been made jointly by Doctor Rogoff and myself. A report summarizing the results presented in this paper was sent to the Committee September 28, 1918—G. N. S.

such different methods were employed. It is all the more notable that in none of the experiments was any evidence obtained that the rate of output was sensibly modified as compared with the initial rate. This result constitutes a new and striking illustration of the fact dwelt upon in previous papers (1) that the output of epinephrin is relatively stable and not easily influenced experimentally.²

The range of epinephrin output per kilogram per minute is, as determined by us, surprisingly narrow, not only in animals of the same species but in different groups. We have had the opportunity of determining the output in a baboon, weighing 2.51 kgm. It worked out at 0.0002 mgm. per kgm. per minute. The average found by us in cats, as assayed by intestine and uterus segments, is 0.00025 mgm. per kgm. per minute, and it is not very different in the dogs examined. Statements in the literature (6) to the effect that in shock conditions the output is much increased, even it may be to more than thirty-two times the initial output, are based upon faulty experimentation, the method of bio-assay adopted not being properly applied. The tracings accompanying the paper cited do not indicate in what manner the test objects (rabbit intestine "strips," meaning probably segments) were subjected to the action of the bloods. No quantitative estimations of the amount of epinephrin liberated per unit of time either before or after shock was induced are given. Nor is there any statement of the concentrations of epinephrin in the blood from which such estimations could be made. We are unable to follow the statement that shock can be induced without any lowering of blood pressure and "that in cases

² The fact that the output of epinephrin per minute remains practically the same with very different rates of blood flow through the gland is of itself sufficient to invalidate the contention of Popielski (2) against the existence of secretory nerves for the adrenals. He asserts that the result of stimulation of the splanchnics on the liberation of epinephrin is merely due to washing out already accumulated epinephrin through the increased blood flow. Since we have shown that increased output does not as a matter of fact accompany increased blood flow, this argument falls to the ground. It is astonishing that it should ever have been put forward. The complete abolition of the output of epinephrin by section of the nerves of the gland is certainly not accompanied by any marked diminution in the blood flow. We have not noticed that it is materially altered. And Burton-Opitz and Edwards (3) saw no definite increase in the adrenal blood flow during stimulation of the peripheral end of the splanchnic when a change of general arterial pressure was prevented. Popielski's statement that manipulation of the adrenal does not liberate epinephrin unless the gland is afterwards flushed out with blood, as by stimulating the splanchnics, is manifestly erroneous, as abundantly demonstrated in our own work (4), (5).

of shock accompanied by high blood pressure, the blood contains relatively a large amount of epinephrin." Some writers have asserted that in shock the store of epinephrin in the suprarenals is diminished. Apart from the fact that the methods used for the assay have ordinarily been inadequate, and that account has not been taken of the effect of anesthetics upon the store, there is no necessary relation between diminished store and increased output, as we have more than once pointed out (7).

A few of the protocols and some of the tracings used for the epinephrin assay in our experiments follow. The epinephrin was assayed by the method introduced by one of us (rabbit intestine segments) (8). In most cases the rabbit uterus segment was also employed to corroborate and sometimes to render more delicate the assay made on the intestine. As we have pointed out in previous papers, it is essential in order to obtain the best results from these methods of bio-assay, that numerous tests should be made with each blood. As a general rule it is advantageous to approximate to the epinephrin concentration in the blood specimen by determining a concentration of adrenalin which is definitely greater and one which is definitely less; then narrowing the interval between the higher and lower concentrations by a sufficient number of observations. It is then not difficult to obtain a concentration of adrenalin which gives a tracing approximately the same as that of the blood. This, however, is not always necessary in order to make a sufficiently close assay, if the higher and lower concentrations are not too far apart.

Condensed protocol

Dog 9. Male. Weight 6.6 kgm.

9.30 a.m. 50 mgm morphine hypodermically

10.45 a.m. Etherized. Put cannulae in trachea, carotid and jugular. Obtained specimen of indifferent blood from jugular

11.05 to 11.30 a.m. Made cava pocket,³ tying abdominal aorta near the bifurcation

³ In all of the experiments the cava pocket was made in a similar manner. The renal vessels and all veins entering the segment of cava between the liver and the point of insertion of the cannula were tied, leaving a pouch into which only the adrenal veins entered. The abdominal aorta was tied near the bifurcation. The cannula was inserted into the lower end of the cava and after pressing the blood out of the segment of cava the pocket was completed by a clamp, just beneath the liver immediately before collection of adrenal blood. After collecting each pair of specimens a fresh cannula was inserted into the pocket.

- 11.30 a.m. Arterial blood pressure 90 mm. of mercury. Now collected adrenal vein blood from the pocket
1st specimen 4.75 grams in 30 seconds (9.6 grams per minute)
2nd specimen 9.6 grams in 60 seconds (9.6 grams per minute)
- 11.35 a.m. Exposed intestines, with occasional manipulation
- 12.30 p.m. Blood pressure 68 mm. of mercury
- 12.32 p.m. Collected adrenal blood from pocket
3rd specimen, 2.35 grams in 30 seconds (4.7 grams per minute)
4th specimen, 7.3 grams in 90 seconds (4.7 grams per minute)
- 12.40 p.m. Replaced intestines in abdomen and fastened abdomen
- 12.45 p.m. Blood pressure 70 mm. of mercury
- 1.10 p.m. Blood pressure 80 mm. of mercury. Again exposed intestines
- 1.25 p.m. Blood pressure 60 mm. of mercury
- 1.45 p.m. Blood pressure 55 mm. of mercury
- 1.50 p.m. Collected adrenal blood from pocket
5th specimen, 2.3 grams in 60 seconds (2.3 grams per minute)
6th specimen, 6.8 grams in 180 seconds (2.3 grams per minute)
- Combined weight of adrenals, 0.848 gram.

The epinephrin assay was made on the 2nd, 4th and 6th adrenal blood specimens. As in all this work, a specimen was collected immediately before each of these (1st, 3rd and 5th), so as to wash the cava segment completely free from any blood entering it during the manipulation connected with the clipping off of the pocket. The flow was not interrupted at all between the collection of the 1st and 2nd, 3rd and 4th or 5th and 6th specimens. After each pair of specimens had been obtained a fresh cannula was inserted into the cava so as to be ready for the next collection. In figures 1 to 6 are reproduced some of the tracings used for the assay. Those of the 6th specimen are given more completely than the others so as to illustrate once for all the manner in which the concentration of epinephrin in the blood is approximated to.

Figure 1 shows that the 2nd specimen, collected before the induction of shock, is notably weaker than the 4th, collected one hour after exposure of the intestines with a blood pressure of 68 mm., and that the 4th specimen is weaker than the 6th, collected two and one-quarter hours after exposure of the intestines, with a blood pressure of 55 mm. or less. This figure is reproduced to emphasize the fact that a qualitative comparison of tracings can never be used to determine whether a change in the rate of output of epinephrin has occurred, a point habitually neglected by some writers on this subject.

Although the much greater inhibition of the intestine segment produced by the 6th specimen than by the 2nd might suggest to a reader

unfamiliar with these methods of assay that the output had been much increased after shock, the assay proved that as a matter of fact there was no change. Figure 2 shows that the 6th specimen (observation 16) is much stronger than 1:3,750,000 adrenalin (observation 12), and stronger than 1:2,500,000 (observation 14). The tracings reproduced in figure 3 demonstrate that the 6th specimen (observation 48) is stronger than 1:1,875,000 adrenalin (observation 50). Figure 3-A shows that it

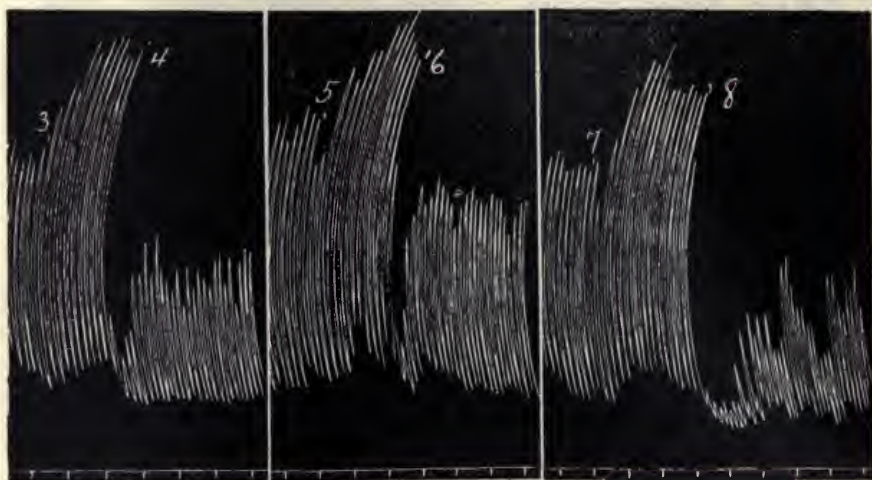


Fig. 1. Intestine tracings. Bloods from dog 9. At 3, 5 and 7 Ringer was replaced by jugular blood and this was replaced at 4 by the 4th adrenal blood specimen (collected after 1 hour of exposure of intestines, blood pressure 68 mm. of mercury); at 6 by the 2nd adrenal blood specimen (collected before exposure of intestines, blood pressure 90 mm. of mercury) and at 8 by the 6th adrenal blood specimen (collected after 2 hours and 20 minutes exposure of intestines, blood pressure 55 mm. of mercury). All the bloods were diluted with 3 volumes of Ringer. As in all the tracings, time is marked in half-minutes. (Reduced to one-half.)

does not differ greatly from 1:1,250,000. The 2nd specimen is shown in figure 4 to be stronger than adrenalin 1:8,750,000. A pair of observations made immediately thereafter (not reproduced) proved that the 2nd specimen was stronger than adrenalin 1:7,500,000. A pair of observations made immediately before those in figure 4 indicated that the concentration of epinephrin in the 2nd specimen did not differ much from 1:6,250,000, and this was confirmed by later observations (not reproduced) on the same intestine segment.

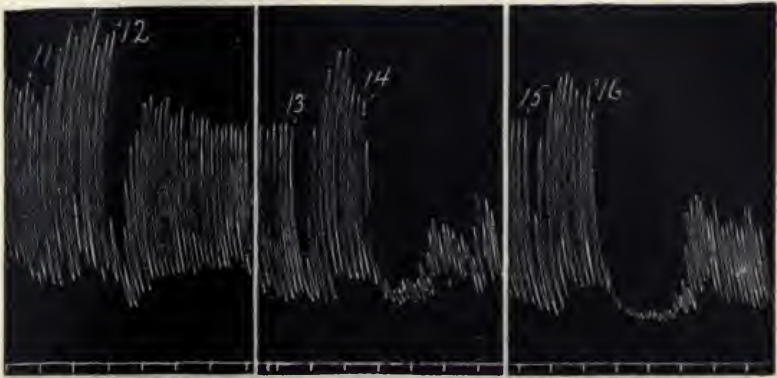


Fig. 2. Intestine tracings. Bloods from dog 9. At 11, 13 and 15 Ringer was replaced by jugular blood and this was replaced at 12 by jugular blood to which had been added adrenalin to make a concentration of 1:3,750,000; at 14 by jugular blood with adrenalin added to make a concentration of 1:2,500,000 and at 16 by the 6th adrenal blood specimen, which is stronger than 1:2,500,000 (from other observations twice as strong). The difference between 14 and 16 appears relatively small simply because the inhibition at 14 is already so great. All the bloods were diluted with 3 volumes Ringer (the adrenalin bloods after adding the adrenalin). (Reduced to one-half.)

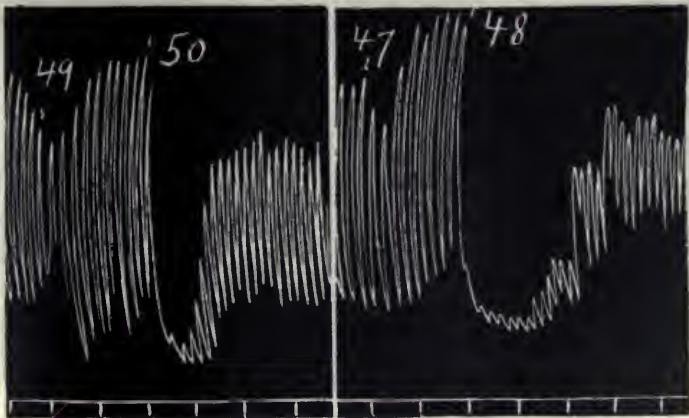


Fig. 3. Intestine tracings. Bloods from dog 9. At 47 and 49 Ringer was replaced by jugular blood and this was replaced at 48 by the 6th adrenal blood specimen and at 50 by jugular blood to which was added adrenalin to make a concentration of 1:1,875,000. The bloods were diluted with 3 volumes Ringer (the adrenalin blood after adding the adrenalin). (Reduced to four-fifths.)

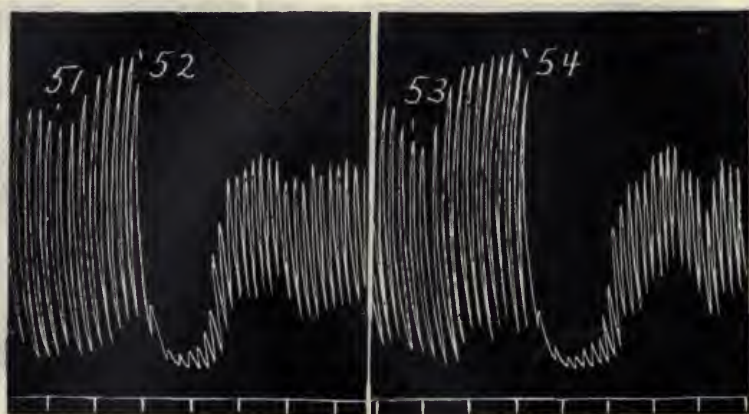


Fig. 3-A. Intestine tracings. Bloods from dog 9. At 51 and 53 Ringer was replaced by jugular blood and this was replaced at 52 by jugular blood with adrenalin added to make a concentration of 1:1,250,000 and at 54 by the 6th adrenal blood specimen. The bloods were diluted with 3 volumes Ringer (the adrenalin blood after adding the adrenalin). (Reduced to four-fifths.)

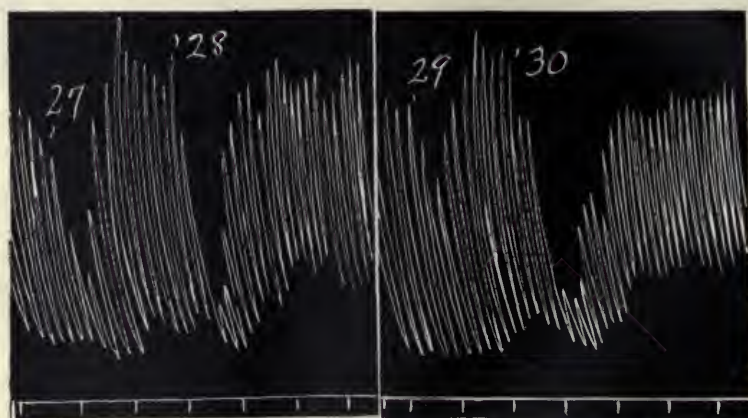


Fig. 4. Intestine tracings. Bloods from dog 9. At 27 and 29 Ringer was replaced by jugular blood and this was replaced at 28 by jugular blood to which was added adrenalin to make a concentration of 1:8,750,000 and at 30 by the 2nd adrenal blood specimen (collected before exposure of intestines). All the bloods were diluted with 3 volumes Ringer (the adrenalin blood after adding the adrenalin). (Reduced to four-fifths.)



Fig. 5. Intestine tracings. Bloods from dog 9. At 39, 41 and 43 Ringer was replaced by jugular blood and this was replaced at 40 by the 4th adrenal blood specimen (collected after 1 hour exposure of intestines, blood pressure 68 mm. of mercury); at 42 by jugular blood to which was added adrenalin to make a concentration of 1:2,500,000 and at 44 by jugular blood with adrenalin added to make a concentration of 1:3,750,000. All the bloods were diluted with 3 volumes Ringer (the adrenal bloods after adding the adrenalin). (Reduced to four-fifths.)

In figure 5 it is demonstrated that the 4th adrenal specimen (observation 40) is decidedly weaker than 1:2,500,000 (observation 42) and somewhat stronger than 1:3,750,000 (observation 44). It was confirmed by two other pairs of observations (not reproduced), one just before and the other just after those recorded in figure 5, that the 4th specimen was not much different from 1:3,750,000, probably a little stronger.

In figure 6 are given samples of uterus tracings showing that the 2nd adrenal specimen produces a greater increase of tone than the indifferent (jugular) blood; the 4th specimen a greater increase than the 2nd and

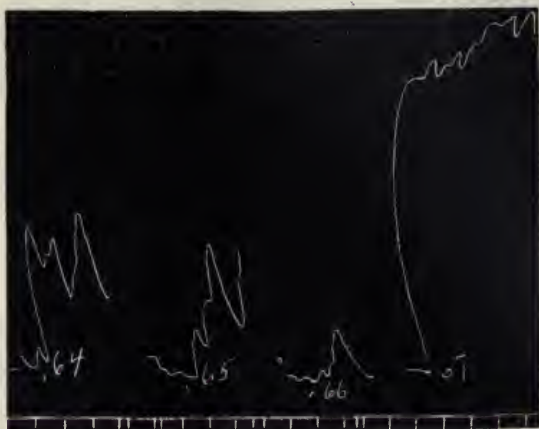


Fig. 6. Uterus tracings. Bloods from dog 9. At 64 Ringer was replaced by the 4th adrenal blood specimen; at 65 by the 2nd adrenal blood specimen; at 66 by jugular blood and at 67 by the 6th adrenal blood specimen. All the bloods were diluted with 2 volumes Ringer. (Reduced to one-half.)

the 6th a much greater increase than the 4th. Again it must be pointed out that the only conclusion which could be drawn from a comparison of the curves in figure 6, taken by themselves, would be that the concentration of epinephrin in the 6th specimen was greater than that in the 4th and the concentration in the 4th greater than that in the 2nd. It would be impossible to give any quantitative value to the differences. We know that in fact the 6th specimen was about three times as strong as the 4th and the 4th less than twice as strong as the 2nd. But this information is only obtainable by a systematic and rather laborious assay. Yet some writers from mere inspection of a few tracings revealing far less striking differences than these shown in figure 6, without any

attempt at quantitative estimation, have ventured to draw far reaching conclusions as to the influence of this or that factor in markedly increasing the rate of output of epinephrin. It may be safely assumed that when a paper does not give the amount of epinephrin liberated per unit of time, before and after the operation of the factor supposed to influence the rate of liberation but merely concludes that the rate has been greatly increased or greatly diminished, it does not contain any real evidence on the question.

Taking the concentration of epinephrin in the second specimen from dog 9 as 1:6,250,000 we get an output of 0.0015 mgm. per minute for the dog, or 0.00023 mgm. per kgm. of body weight per minute. Taking the 4th specimen at 1:3,500,000 we get 0.0013 mgm. per minute for the dog, or 0.0002 mgm. per kgm. per minute. Taking the concentration in the 6th specimen as 1:1,250,000 we get 0.0018 mgm. per minute for the dog, or 0.00027 mgm. per kgm. per minute.

Condensed protocol

- Dog 6.* Weight 6.2 kgm.
- 11.00 a.m. Morphine 25 mgm. hypodermically
 - 1.00 p.m. Etherized. Put cannulae in trachea, carotid and jugular. Made cava pocket
 - 1.50 p.m. Blood pressure 154 mm. of mercury
 - 1.55 p.m. Obtained indifferent blood from jugular. Now collected adrenal blood from the pocket
 - 1st specimen, 7.6 grams in 45 seconds (11.5 grams per minute)
 - 2nd specimen, 22.9 grams in 120 seconds (11.5 grams per minute)
 - Blood pressure during collection of these specimens 123 mm. of mercury
 - 2.00 p.m. Exposed intestines; occasional manipulation
 - 2.07 p.m. Blood pressure 85 mm. of mercury
 - 2.35 p.m. Blood pressure 66 mm. of mercury
 - 2.55 p.m. Blood pressure 63 mm. of mercury. Collected adrenal blood from pocket
 - 3d specimen, 7.4 grams in 60 seconds (7.4 grams per minute)
 - 4th specimen, 16.0 grams in 150 seconds (6.4 grams per minute)
- Combined weight of adrenals 0.905 gram.

In this experiment the epinephrin assay showed that the 2nd adrenal blood specimen (taken before manipulation of the intestines was begun, with a blood pressure of 123 mm. of mercury) had a concentration of 1:6,500,000. The output per minute for the dog was 0.0018 mgm., or 0.0003 mgm. per kgm. of body weight per minute. The 4th specimen collected nearly an hour after manipulation was begun, with blood

pressure of 63 mm. of mercury, had a concentration of 1:3,500,000, corresponding to an output per minute for the dog of 0.0018 mgm., or 0.0003 mgm. per kgm. per minute. A small sample of the tracings used in the assay is reproduced in figure 7, which shows that the 4th specimen (observation 17) had a concentration decidedly greater than 1:4,000,000 (observation 19), but somewhat less than 1:3,000,000 (observation 21). This was confirmed by other observations.

In a third experiment on a dog, weighing 7.25 kgm. (dog 7), a specimen of adrenal blood collected before exposure of the intestines, with a blood

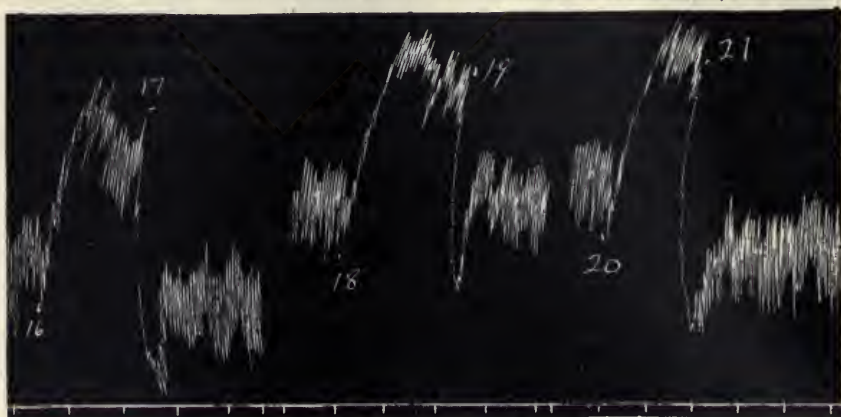


Fig. 7. Intestine tracings. Bloods from dog 6. At 16, 18 and 20 Ringer was replaced by jugular blood and this was replaced at 17 by the 4th adrenal blood specimen (collected after 1 hr. exposure of intestines, blood pressure 63 mm. of mercury); at 19 by jugular blood to which was added adrenalin to make a concentration of 1:4,000,000 and at 21 by jugular blood with adrenalin added to make a concentration of 1:3,000,000. All the bloods were diluted with 2 volumes Ringer (the adrenalin bloods after adding the adrenalin). (Reduced to four-sevenths.)

pressure of 113 mm. of mercury, had a concentration of epinephrin of 1:1,800,000, giving an output of 0.0018 mgm. per minute for the dog, or 0.00025 mgm. per kgm. per minute. A specimen, collected two hours after exposure of the intestines, was assayed at 1:1,250,000 epinephrin, corresponding to an output per minute for the dog of 0.0014 mgm., or 0.0002 mgm. per kgm. per minute. The blood pressure ten minutes before collection of this specimen was 72 mm. of mercury and at the end of collection it was 48 mm. of mercury. The flow during collection of the last specimen was probably too small, considering that

the concentration was approaching the possible maximum to allow the calculated output quite to equal the output before shock was induced.

Condensed protocol

Dog 2. Male. Weight 7.3 kgm.

- 9.00 a.m. Morphine 50 mgm. hypodermically
10.50 a.m. Etherized. Put cannulae into trachea, carotid and jugular. Obtained indifferent blood from jugular
11.25 a.m. Started artificial respiration
11.45 a.m. Loose ligature placed around inferior cava, in thorax. Made cava pocket
12.40 p.m. Blood pressure 140 mm. of mercury.
12.50 p.m. Blood pressure 100 mm of mercury. Now collected adrenal blood from the pocket
1st specimen, 7.85 grams in 60 seconds (7.85 grams per minute)
2nd specimen, 16.1 gram in 120 seconds (8.0 grams per minute)
1.00 p.m. Partial occlusion of cava with occasional release, practiced at intervals for one and one-half hours. During this period the blood pressure gradually fell to 68 mm. of mercury at 2.30 p.m.
2.45 p.m. Collected adrenal blood from pocket
3rd specimen, 4.2 grams in 60 seconds (4.2 grams per minute).
4th specimen, 11.35 grams in 180 seconds (3.8 grams per minute).
5th specimen, 8.85 grams in 180 seconds (3.0 grams per minute)
Combined weight of adrenals 1.2 grams.

The second adrenal blood specimen (from dog 2) collected before occlusion of the cava, with blood pressure 105 mm. of mercury, was assayed at 1:6,500,000 corresponding to an output of 0.0012 mgm. per minute for the animal, or 0.00017 mgm. per kgm. per minute. The 4th specimen, collected after shock had been induced, contained 1:2,700,000 epinephrin, giving an output of 0.0014 mgm. per minute for the dog, or 0.00019 mgm. per kgm. per minute. The 5th specimen, collected with a blood pressure of 68 mm. of mercury, had a concentration of 1:2,100,000 corresponding to an output of 0.0014 mgm. per minute for the animal, or 0.00019 mgm. per kgm. per minute.

Condensed protocol

Dog 1. Female. Weight 11.3 kgm.

- 9.00 a.m. Morphine 100 mgm. hypodermically
10.45 a.m. Etherized. Put cannulae in trachea, carotid and jugular. Made cava pocket

- 11.30 a.m. Blood pressure 100 mm. of mercury. Now collected adrenal blood from pocket
1st specimen, preliminary specimen
2nd specimen, 17.4 grams in 30 seconds (34.8 grams per minute)
- 11.45 a.m. Bled from carotid (about 250 cc. blood withdrawn). Blood pressure just after hemorrhage, 60 mm. of mercury. Collected a preliminary specimen, then
3d specimen, 9.5 grams in 60 seconds
- 11.50 a.m. Closed abdomen with clamps
- 12.00 to 12.30 p.m. Pulse feeble and rapid; respirations very rapid (180 per minute)
- 1.50 p.m. Collected adrenal blood from pocket
4th specimen, 3.35 grams in 30 seconds (6.7 grams per minute)
5th specimen, 11.45 grams in 150 seconds (4.6 grams per minute)
Blood pressure just after collection of 5th specimen 40 to 50 mm. of mercury
- Combined weight of adrenals 1.8 grams.

In this animal the 2nd adrenal blood specimen, obtained before hemorrhage, with the blood pressure at 100 mm. of mercury, was assayed at 1:18,000,000 epinephrin. The rate of flow from the adrenals was large although no unligated small vein connected with the cava pocket was found *post mortem*. This accounts for the exceptionally low concentration of epinephrin. The output of epinephrin per minute for the animal for this specimen was 0.002 mgm. or 0.00018 mgm. per kgm. of body weight per minute. The 3rd specimen, collected immediately after hemorrhage, with the blood pressure at 60 mm. of mercury, had a concentration of 1:3,300,000 of epinephrin, corresponding to an output of 0.0029 mgm. per minute for the dog, or 0.00025 mgm. per kgm. per minute. The 5th adrenal blood specimen, collected two hours after the hemorrhage, the blood pressure being 50 mm. of mercury at the beginning and 40 mm. of mercury at the end of collection, had a concentration of epinephrin of 1:1,500,000. This corresponds to an output of 0.003 mgm. per minute for the animal, or 0.00025 mgm. per kgm. per minute. Samples of the tracings used for the assays are reproduced in figures 8 to 12.

Figure 8 shows that at this stage the second adrenal specimen (observation 3) caused no definite inhibition of the intestine segment, which was not sensitive enough to detect the concentration of epinephrin contained in it, although later on it was assayed at 1:18,000,000. The 3rd specimen caused a good inhibition (observation 5). It was assayed in other observations at 1:3,300,000. This is an excellent illustration of the danger of drawing quantitative conclusions from quali-

tative experiments. No doubt the inhibition in observation 5 was produced by a concentration of epinephrin five times as great as that which failed to cause inhibition in observation 3. But similar observations have sometimes been erroneously interpreted as indicating that a great "outburst" of epinephrin must have occurred when the specimen giving the positive reaction was being collected. In the present instance, as the assay showed, the rate of output of epinephrin was practically the same for both specimens. Figure 9 demonstrates that the 2nd adrenal specimen contained enough epinephrin to be detected and ap-

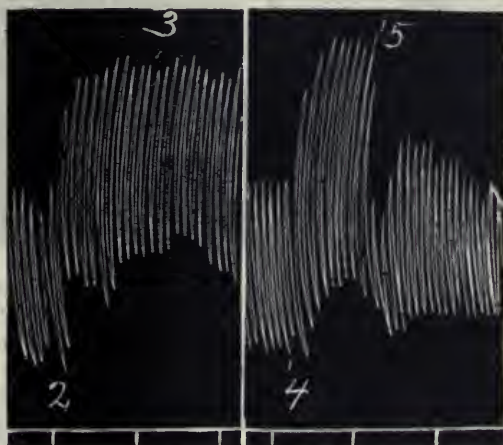


Fig. 8. Intestine tracings. Bloods of dog 1. At 2 and 4 Ringer was replaced by indifferent blood and this was replaced at 3 by the 2nd adrenal blood specimen (collected before hemorrhage, blood pressure 100 mm. of mercury) and at 5 by the 3rd adrenal blood specimen (collected a few minutes after hemorrhage, blood pressure 60 mm. of mercury). The bloods were diluted with 3 volumes Ringer. (Reduced to two-thirds.)

proximately assayed by the same rabbit intestine segment when it had become more sensitive to epinephrin. The concentration is obviously less than 1:16,500,000 (observation 43) and greater than 1:20,000,000 (observation 45). These observations were confirmed by others and the concentration worked out at approximately 1:18,000,000.

In figure 10 it is shown that the concentration of the 3rd adrenal specimen is slightly greater than 1:3,400,000. This was confirmed by other observations. It was taken finally at 1:3,300,000. Figure 11 shows that the concentration of the 5th specimen is not very different from 1:1,650,000 and figure 12, that it is considerably less than 1:1,250,000. It was finally taken as 1,500,000.

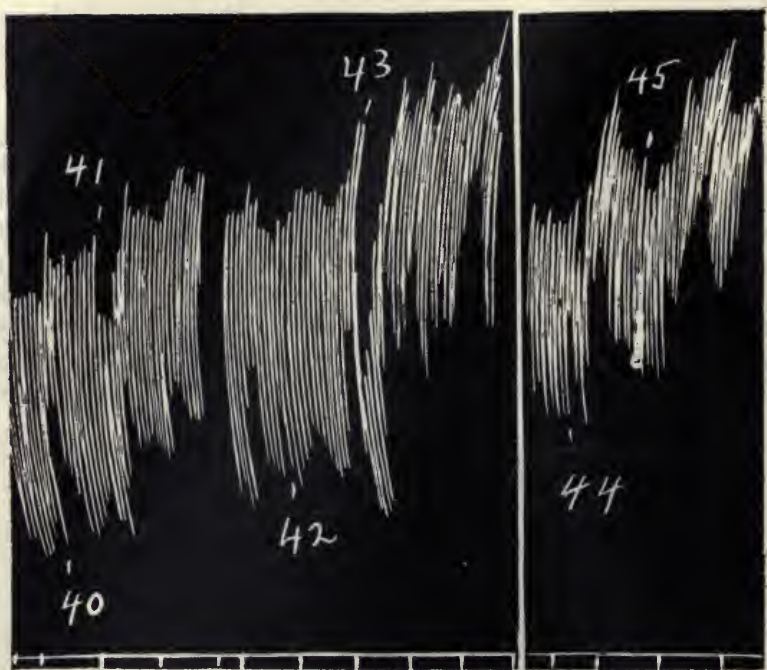


Fig. 9. Intestine tracings. Bloods from dog 1. At 40, 42 and 44 Ringer was replaced by indifferent blood and this was replaced at 41 by the 2nd adrenal blood specimen (collected before hemorrhage); at 43 by indifferent blood to which was added adrenalin to make a concentration of 1: 16,500,000 and at 45 by indifferent blood, with adrenalin added to make a concentration of 1: 20,000,000. All of the bloods were diluted with an equal volume of Ringer (the adrenalin bloods after adding the adrenalin).

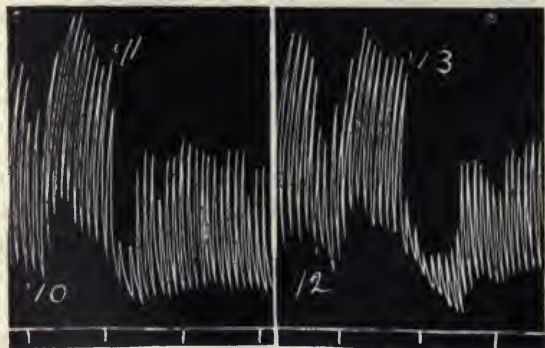


Fig. 10. Intestine tracings. Bloods from dog 1. At 10 and 12 Ringer was replaced by indifferent blood and this was replaced at 11 by indifferent blood to which was added adrenalin to make a concentration of 1: 3,400,000 and at 13 by the 3rd adrenal blood specimen (collected a few minutes after hemorrhage). All the bloods were diluted with 3 volumes Ringer (the adrenalin blood after adding the adrenalin). (Reduced to two-thirds.)

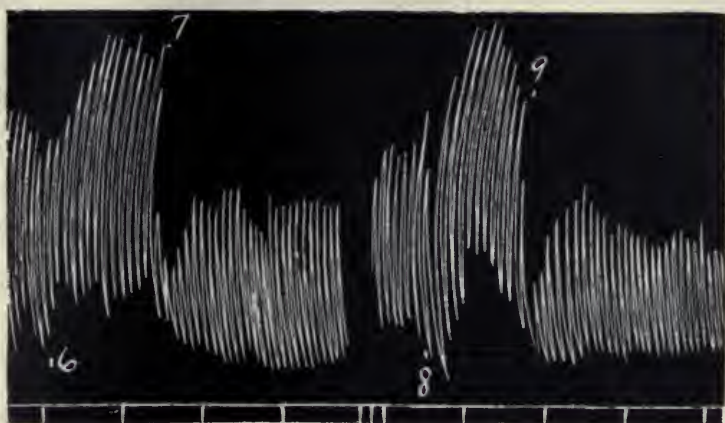


Fig. 11. Intestine tracings. Bloods from dog 1. At 6 and 8 Ringer was replaced by indifferent blood and this was replaced at 7 by the 5th adrenal blood specimen (collected 2 hours after hemorrhage, blood pressure 40 to 50 mm. of mercury) and at 9 by indifferent blood to which was added adrenalin to make a concentration of 1:1,650,000. The bloods were diluted with 3 volumes Ringer (the adrenalin blood after adding the adrenalin). (Reduced to two-thirds.)

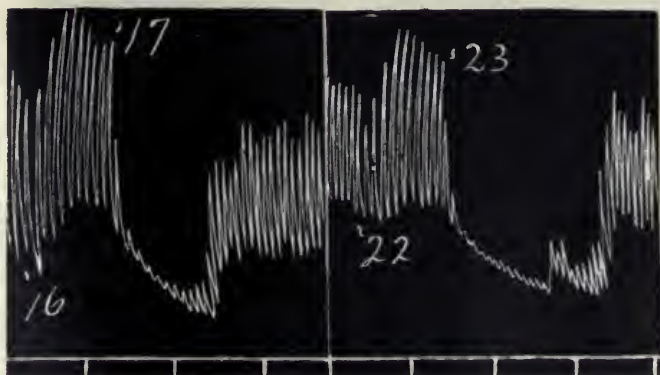


Fig. 12. Intestine tracings. Bloods from dog 1. At 16 and 22 Ringer was replaced by indifferent blood and this was replaced at 17 by the 5th adrenal blood specimen and at 23 by indifferent blood to which was added adrenalin to make a concentration of 1:1,250,000. All the bloods were diluted with 3 volumes Ringer (the adrenalin blood after adding the adrenalin). (Reduced to four-fifths.)

Condensed protocol

- Cat 4.* Male. Weight 3.255 kgm. Urethane anesthesia.
- 3.00 p.m. Put cannulae in trachea, carotid and femoral vein. Obtained indifferent blood from femoral vein. Started artificial respiration. Made cava pocket
- 3.40 p.m. Blood pressure before opening abdomen 120 mm. of mercury. Collected adrenal blood from the pocket
 1st specimen, 4.85 grams in 20 seconds (14.5 grams per minute)
 2nd specimen, 6.65 grams in 30 seconds (13.3 grams per minute)
 3d specimen, 6.4 grams in 30 seconds (12.8 grams per minute)
 Blood pressure at beginning of collection of 3d specimen, 104 mm. of mercury
- 3.45 p.m. Blood pressure after collection of 3d specimen, 70 mm. of mercury. Closed abdomen with clips
- 3.50 p.m. Blood pressure 105 mm. of mercury
 Injected 1½ grams Witte's peptone (in 10 per cent solution) *via* external jugular vein
 Blood pressure at end of peptone injection, 70 mm. of mercury
- 4.10 p.m. Collected adrenal blood from the pocket
 4th specimen, 3.65 grams in 30 seconds (7.3 grams per minute)
 5th specimen, 9.95 grams in 120 seconds (5.0 grams per minute)
 6th specimen, 11.20 grams in 180 seconds (3.7 grams per minute)
 Blood pressure after collection of 6th specimen, 60 mm. of mercury
- 4.17 p.m. Obtained indifferent blood from external jugular vein. (Label this "peptone indifferent blood")
- 4.40 p.m. Collected adrenal blood from pocket
 7th specimen, 0.72 gram in 60 seconds
 8th specimen, 0.68 gram in 60 seconds
 Blood pressure during collection of 8th specimen, 28 mm. of mercury
 Combined weight of adrenals 0.60 gram.

The 3rd, 5th and 8th adrenal blood specimens from cat 4 were carefully assayed, that is, one specimen collected before injection of peptone and two specimens after injection. For indifferent blood the "peptone blood" collected from the jugular was used in the assay of the 5th and 8th specimens. It was tested, however, against the indifferent blood obtained before injection of peptone and found not to differ materially from it as regards its action on the segments. The 3rd adrenal specimen assayed at 1:14,000,000 to 1:15,000,000 epinephrin, corresponding to an output of 0.0009 mgm. per minute for the animal, or 0.00027 mgm. per kgm. per minute. The blood pressure was 104 mm. of mercury at the beginning of collection of the 3rd specimen. The relatively low concentration is associated with a high rate of flow through the adrenals. No leak into the cava pocket from any other source could be detected *post mortem*. The 5th adrenal specimen, taken

twenty minutes after injection of the peptone, with blood pressure about 60 mm. of mercury, had a concentration of 1:6,500,000, giving an output of 0.0008 mgm. of epinephrin per minute for the cat, or 0.00025 mgm. per kgm. of body weight per minute. The 8th specimen was assayed at 1:850,000, giving an output of 0.0008 mgm. epinephrin per minute for the cat, or 0.00025 mgm. per kgm. per minute. Although the concentration in this specimen approached the possible maximum concentration, owing to the small blood flow through the adrenals,

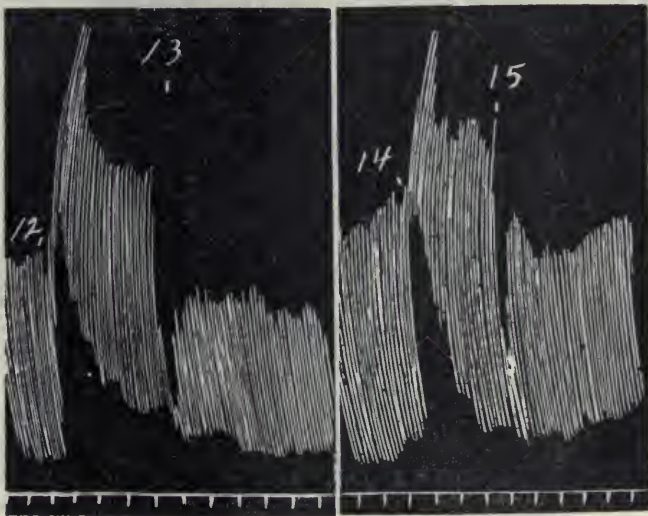


Fig. 13. Intestine tracings. Bloods from cat 4. At 12 and 14 Ringer was replaced by femoral vein blood and this was replaced at 13 by femoral vein blood to which was added adrenalin to make a concentration of 1:14,000,000 and at 15 by the 3rd adrenal blood specimen (collected before injection of peptone, blood pressure 104 mm. of mercury). All the bloods were diluted with 3 volumes Ringer (the adrenalin blood after adding the adrenalin). (Reduced to four-fifths.)

associated with the low blood pressure at the time of collection (28 mm. of mercury), the curves obtained permitted a good assay. The results were confirmed on the uterus segments. Some of the intestine segment tracings are reproduced in figures 13 to 16. Figure 13 indicates that the concentration of the 3rd specimen is somewhat less than 1:14,000,000. In other tracings it was shown that 1:16,500,000 was markedly weaker than the 3rd specimen, while 1:14,000,000 was only slightly stronger. In figure 14 it is demonstrated that the 5th speci-

men was weaker than 1:5,700,000. Other tracings showed that it was stronger than 1:7,000,000. In figures 15 and 16 are reproduced some of the tracings used to assay the 8th specimen. Figure 15 shows that 1:1,400,000 (observation 55) was much too weak for the specimen and that 1:700,000 (observation 57) was too strong. From figure 16 it is clear that the specimen was stronger than 1:1,000,000.

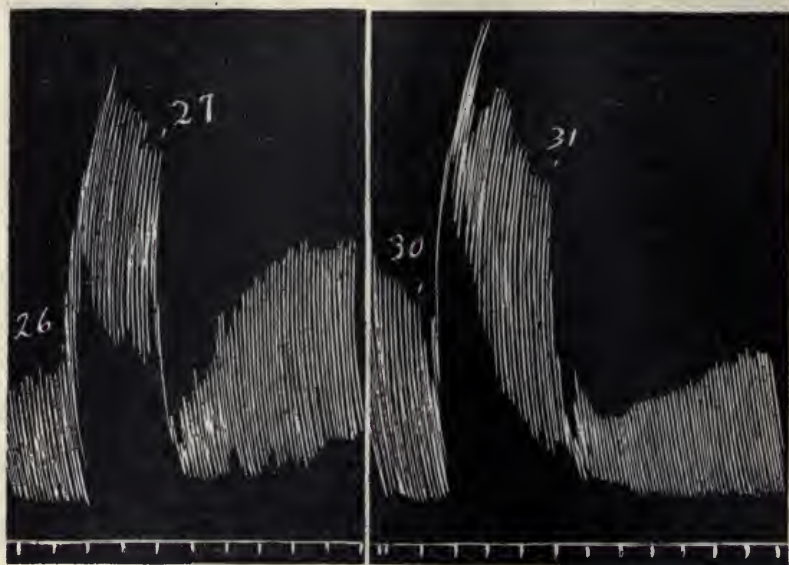


Fig. 14. Intestine tracings. Bloods from cat 4. At 26 and 30 Ringer was replaced by jugular blood and this was replaced at 27 by the 5th adrenal blood specimen (collected 20 minutes after injection of peptone) and at 31 by jugular blood to which was added adrenalin to make a concentration of 1:5,700,000. All the bloods were diluted with 3 volumes Ringer (the adrenalin blood after adding the adrenalin). (Reduced to four-fifths.)

The results of this investigation may be summed up in a sentence. In none of our experiments has evidence been obtained that any sensible change occurs in the rate of output of epinephrin from the adrenals in conditions of continued low blood pressure.

The only way in which we have hitherto been able to bring about an unequivocal increase in the epinephrin output (apart, of course, from that due to stimulation of the peripheral end of the splanchnic

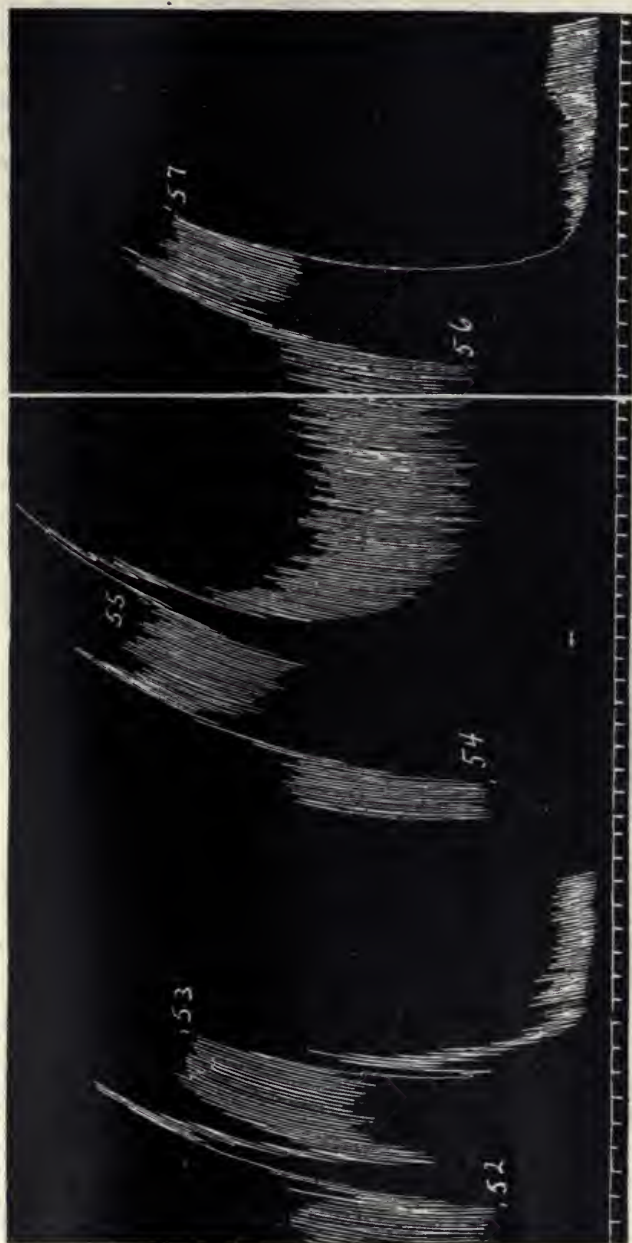


Fig. 15. Intestine tracings. Bloods from cat 4. At 52, 54 and 56 Ringer was replaced by jugular blood and this was replaced at 53 by the 8th adrenal blood specimen (collected $\frac{1}{2}$ hour after peptone injection, blood pressure 28 mm. of mercury); at 55 by jugular blood to which was added adrenalin to make a concentration of 1:1,400,000 and at 57 by jugular blood with adrenalin added to make a concentration of 1:700,000. All the bloods were diluted with 6 volumes Ringer (the adrenalin bloods after adding the adrenalin.) (Reduced to two-thirds.)

nerve) is by the administration of strychnine or the intravenous injection of small quantities of concentrated solutions of salts (sodium carbonate), which cause a general excitation of the spinal cord. We purposely produced such an excitation in the hope of stimulating among other centers that which is associated with the secretion of epinephrin and is situated in the upper part of the thoracic cord (9). For this reason we have hitherto investigated the action of strychnine only in

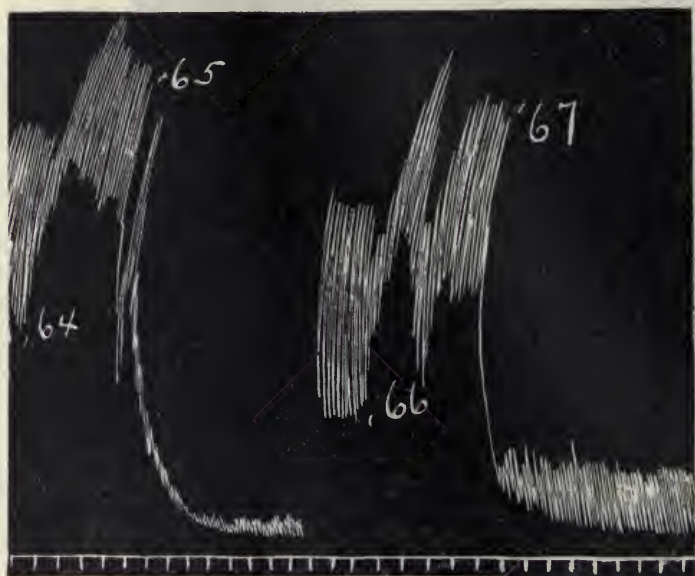


Fig. 16. Intestine tracings. Bloods from cat 4. At 64 and 66 Ringer was replaced by jugular blood and this was replaced at 65 by the 8th adrenal blood specimen and at 67 by jugular blood to which was added adrenalin to make a concentration of 1:1,000,000. All the bloods were diluted with 10 volumes Ringer (the adrenalin blood after adding the adrenalin). (Reduced to four-fifths.)

doses somewhat higher than the ordinary therapeutic doses, and have obtained a very marked increase of the rate of epinephrin output, both in the dog and cat, up to ten times the initial output. Asphyxia was excluded by artificial respiration. The effect of strychnine is not transient and may outlast for a considerable time the convulsant action, when such is present. The maximum increase is not necessarily found in specimens of adrenal blood taken immediately after the appearance of the symptoms of strychnine action on the cord. Thus, in one experi-

ment the output of epinephrin in blood collected immediately after the onset of the symptoms was shown to be three times the initial output, whereas one hour later it was four times the initial output. In another experiment the output was doubled with the onset of the strychnine symptoms but more than an hour later, when the symptoms had apparently completely subsided, the output was three times the initial output. In a third experiment, on a deeply anesthetized animal in which the dose of strychnine employed caused no convulsions, the epinephrin output was definitely increased in a blood specimen taken a few minutes after the injection of the strychnine, but the increase was twice as great in a specimen collected a little more than an hour thereafter. It is not always the case, however, that the later specimens show a greater increase than those collected soon after the administration of strychnine. In one experiment, for example, the output soon after the development of the strychnine symptoms was ten times the initial output, while a specimen taken three-quarters of an hour later showed six or seven times the initial output. Of course, the extent of the increase varies in different experiments. The subject is being investigated further, especially the action of smaller doses, and the details will be published in a subsequent paper. The possibility that the stimulating effect of strychnine upon the epinephrin output may enter as a factor into some of the pharmacological and therapeutic actions of the drug is obvious, but we have at present no evidence on this point.

SUMMARY

The rate of output of epinephrin in dogs and cats, after the blood pressure had been permanently lowered by exposure and manipulation of the intestines, by partial occlusion of the inferior vena cava, by hemorrhage and by "peptone" injection, was found to be the same as before the lowering of the blood pressure, within the limits of error of the methods used for assaying the epinephrin.

A marked increase in the rate of output of epinephrin was produced by strychnine.

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THE RELATION OF THE ADRENALS TO PIQÛRE HYPER- GLYCEMIA AND TO THE GLYCOGEN CONTENT OF THE LIVER

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PART I. THE RELATION OF THE ADRENALS TO PIQÛRE HYPERGLYCEMIA

We have recently (1) studied the question whether the epinephrin secretion of the adrenals is indispensable for the production of certain experimental hyperglycemias. The majority of previous investigations have suffered from the defect that they were carried out, if not on practically moribund animals, at least on animals still under the effects of a serious operation. This undoubtedly is the chief reason for the astonishing lack of uniformity in the results. Working with animals (cats) in which the epinephrin secretion was abolished or reduced to an insignificant fraction of the normal by removal of one adrenal and section of the nerves of the other (an operation which does not preclude the continued life of the animal in good health), we were able to show that two forms of experimental hyperglycemia—that produced by ether and that produced by asphyxia—are as readily obtained in the absence of epinephrin secretion as when the adrenals have not been interfered with. We purposely reserved the question of the relation of the adrenals to piqûre hyperglycemia for further investigation since it is the form which is commonly supposed to be more closely associated than any other with the activity of the adrenal medulla. In his earlier work Kahn (2) was unable to find evidence of increased epinephrin liberation following piqûre. His later statement (3) that the piqûre causes an augmented epinephrin liberation, even if it were well founded, would by no means settle the question. For his observations furnish no evidence that the quantity liberated is at all comparable to the quantity required to produce the typical adrenalin hyperglycemia and glycosuria when adrenalin is artificially injected. But in fact Kahn's ex-

periments do not show that the output of epinephrin is at all influenced by the sugar puncture. Deductions from the relative depth of the coloration produced by chromium salts in the adrenal medulla have little or no quantitative value. And such estimations as he made by the L wen method are vitiated by the fact that he took no account of changes in the rate of blood flow through the adrenals in the period for which the animal was allowed to survive following the piq re. An increase in epinephrin concentration in the adrenal vein blood shown by the perfused frog preparation could not be interpreted as an increase in the output of epinephrin per unit of time unless it were known that the blood flow through the adrenals had not been proportionately diminished after the puncture, as compared with the flow during collection of the comparison samples before the puncture. Kahn did not even collect pure adrenal vein blood, but drew off blood from the inferior cava. The difference in vasoconstricting power of different samples of serum or defibrinated blood is also a factor which detracts greatly from the value of such estimations on blood-vessel preparations.

It has not been sufficiently recognized by some of the other investigators who have published experiments purporting to show an increased rate of liberation due to this or that factor that certain indispensable conditions must be fulfilled if the comparative tests are to have any quantitative value. When blood from the adrenals is withdrawn and then tested on such objects as rabbit intestine and uterus segments, it is practically always the concentration of epinephrin in the liquid which is estimated, since the concentration of adrenal n added to an indifferent specimen of the same blood which will give an effect equal to that of the adrenal specimen is determined. The condition of the segment in all observations which are to be compared must of course be approximately the same, as we have repeatedly pointed out in other papers. The concentration as thus estimated gives no information as to the rate of liberation of epinephrin per unit of time unless the mean rate of blood flow through the adrenals during collection of the given specimen is known.

When deductions are made in regard to the rate of liberation of epinephrin from experiments on test objects *in situ* it is of course just as necessary to see that in any observations compared the test object is in the same condition as regards reactivity, the rate of the blood flow through it, etc. For example, von Anrep (4) states that if the nerves of a hind limb or kidney be cut, these denervated parts respond to stimulation of afferent nerves (central end of sciatic) by a vasoconstrict-

tion if the adrenals have not been interfered with, but only show the initial passive dilatation due to the rise of blood pressure if the adrenals have been extirpated. He draws the conclusion that stimulation of the sciatic reflexly increases the rate of liberation of epinephrin. But granting that the characteristic vasoconstriction can only be elicited with intact adrenals (and von Anrep's careful work seems to leave little doubt that this is a fact) this does not at all prove that during stimulation of the nerve more epinephrin is being poured into the blood per unit of time than without stimulation, for during stimulation of the sciatic the condition of the test object is greatly altered. The rise of blood pressure must necessarily increase the rate of blood flow through the denervated region. Usually the increase will be very considerable. With the adrenals intact and steadily discharging epinephrin at the normal rate, this means that the amount of epinephrin passing per unit of time through the vascular tract in question is abruptly and markedly augmented. If such denervated areas are as sensitive to epinephrin as is claimed, they may be expected to respond to this increase in the amount of epinephrin traversing them, even if no change whatever has taken place in the rate of its liberation from the adrenals. This reaction, accordingly, is not at all out of harmony with our observations made by a more direct method; namely, collection of adrenal vein blood during and without afferent nerve stimulation, and testing of the blood specimens on rabbit intestine and uterus segments. If afferent stimulation really causes such a great increase in the output of epinephrin as some authors believe, we ought to have been able to capture some of this epinephrin-rich blood coming from the adrenals, but we found no difference in the output in samples collected in equal times with and without afferent nerve stimulation (5). It is obvious that if the phenomenon studied by von Anrep is due to passage through the denervated region of an increased amount of blood with the ordinary content of epinephrin, it will necessarily be abolished by excision of the adrenal or by clipping of the adrenal veins, just as if it were due to a greatly augmented output of epinephrin produced reflexly. All that can be deduced from the fact that after elimination of the adrenals the vasoconstriction in the denervated part is absent, is that epinephrin was being given off from the adrenals, a fact which is undisputed.

We have shown (6) that as a matter of fact an increase in the amount of blood supplied to a test object *in situ* (denervated eye) without change in the concentration of the epinephrin, is associated with an increased epinephrin reaction, or with the appearance of a positive reaction where

none was elicited with the previous rate of blood flow. For example, if a strength and duration of stimulation of the peripheral end of one or of both splanchnics be sought which will just fail to cause dilatation of the pupil of the denervated eye, a definite dilatation will in general be obtained when certain alternative arterial paths are occluded at the proper time, so as to allow more of the epinephrin-containing blood to pass to that eye. The concentration of epinephrin in the blood will, of course, not be affected. A precisely similar result is obtained with artificially introduced adrenalin. Here it would be obviously absurd to say that the pupil reaction had been elicited because the rate of liberation of epinephrin from the adrenals or the rate of injection of adrenalin had been increased by clamping alternative paths at a time when the liberation or injection had been already completed.

Another, perhaps less important, factor may be involved in von Anrep's experiment, which likewise he has not taken into account. The reflex vasoconstriction associated with stimulation of the sciatic may be expected to cause, for a short time at least,—and this is all that concerns us,—a slackening in the flow through the splanchnic area and therefore a diminution in the quantity of blood coming to the inferior cava and mingling with the adrenal blood.¹ The epinephrin secretion proceeding unchanged at the normal rate, the concentration of epinephrin in the blood entering and leaving the heart will thus be increased, so that not only will the denervated region receive a much augmented quantity of blood, but the concentration of epinephrin in this blood may be greater than before the nerve stimulation without any increase having taken place in the output of epinephrin per unit of time.

Von Anrep himself performed an experiment, which we believe proves conclusively that the effects observed by him are not due to augmented epinephrin secretion but to a redistribution of the blood containing epinephrin given off at the previous steady rate. He says:

¹ The observations of Edwards (7) on the compensatory increase of blood flow through the head and limbs during stimulation of the splanchnic are not out of harmony with the supposition that stimulation of the central end of the sciatic may cause an increase of concentration of epinephrin in the blood of the inferior cava in the manner suggested without any change having occurred in the rate of liberation of epinephrin from the adrenals. For we do not know that a similar compensation occurs, or that it occurs as promptly with stimulation of the central end of a peripheral nerve trunk, which may cause vasoconstriction in the head and limbs also, as when the peripheral end of a splanchnic is stimulated.

It is important to note that if one splanchnic nerve is intact while the suprarenal on the other side is extirpated, stimulation of the splanchnic nerve on the side of the extirpated suprarenal may still cause constriction of the denervated limb. Only after the other splanchnic nerve is cut does this constriction disappear and the limb react passively to the changes of blood pressure.

His interpretation of this result is that

this is due to the fact that stimulation, even of the peripheral end (of the splanchnic), excites a certain number of afferent nerves, so that there may be a reflex excitation of the suprarenal of the other side through the intact splanchnic nerve.

There is no evidence that stimulation of the peripheral end of the splanchnic nerve can affect reflexly the rate of epinephrin secretion from the other adrenal, and we have good evidence against it. The true explanation, we believe, is that the liberation of epinephrin is proceeding steadily at a substantial rate from the adrenal with intact splanchnic and blood containing a concentration of epinephrin corresponding to this discharge is passing steadily through the denervated region as through the rest of the vascular system. Whatever influence it is exerting cannot of course be revealed till some change occurs in the concentration or in the amount passing through per unit of time. When the splanchnic on the side of the extirpated adrenal is stimulated, there is no increase in the rate of liberation of epinephrin from the remaining adrenal but the vasoconstriction in the splanchnic area causes a rise of arterial pressure which is straightway reflected in an increased flow of the epinephrin-containing blood through the denervated area. This area naturally responds, granting that such vascular regions are as sensitive to epinephrin as von Anrep assumes, by a vasoconstriction to the larger amount of epinephrin offered to it.

The similar reactions elicited by asphyxia, and obtained not at all or in smaller degree after elimination of the adrenals, in like manner afford no evidence in favor of a greatly augmented output of epinephrin, certainly no evidence which can be set up against such direct tests as we have made with the actual adrenal vein blood (10). In the case of asphyxia an additional and a formidable complication is introduced in such indirect experiments as those of von Anrep by the fact that asphyxia may be expected to alter the reactivity of the test object to epinephrin. Unless this factor is controlled, it is impossible to say that an increased reaction is due to an augmented liberation of epinephrin and not to an increased sensitiveness of the test object to such amounts as were already present.

When we have used as test objects organs or tissues *in situ* we have endeavored to render the observations to be compared really comparable by collecting the blood to be tested in the cava pocket and only releasing it when the test object was again approximately under the original conditions. For instance, in determining whether stimulation of afferent nerves caused any change in the rate of liberation of epinephrin, we collected blood in the pocket for a given time, then released it and noted the effect on the eye and blood pressure. Then blood was collected for the same length of time during nerve stimulation. In many of our experiments the splanchnic area and hind limbs were purposely tied off, so that the reflex rise of blood pressure was usually negligible. But where the nerve stimulation produced any considerable rise of blood pressure the stimulation was stopped some time before the opening of the pocket so that the tissues of the eye-ball and the blood vessels might, as far as practicable, have the opportunity of reverting to the same conditions as regards rate of blood flow, etc., as were present in the comparison observation. Only after this interval was the epinephrin-containing blood in the pocket released. In our observations on asphyxia a longer interval was allowed to permit the blood pressure to return to normal, the respiratory movements to diminish and the asphyxial products, to some extent at least, to disappear. We never supposed that it was permissible to use in one observation an asphyxiated test object and in the comparison observation the same object with unobstructed respiration; or to assume that if there was any difference in the reactions it must be due to a difference in the rate of output of epinephrin, the condition of the test object itself being of no moment.

Certain reactions (especially acceleration) of the heart, when isolated from extrinsic nervous influence by section of the vagi and excision of the stellate ganglia, have also been supposed to prove that the rate of output of epinephrin from the adrenals is greatly increased reflexly by stimulation of afferent nerves. It is not difficult, however, to see that the results of the study of these reactions by von Anrep and others must be interpreted precisely in the same way as the results on the denervated vascular areas, and yield no better evidence of reflexly augmented epinephrin secretion than the vascular reactions do.

Thus von Anrep (8), starting with the demonstration that stimulation of the peripheral end of the cut splanchnics is followed by the characteristic heart reactions if the adrenals are present, but not if they have been removed, is led to the generalization that

every rise of blood pressure brought about by the agency of the nervous system involves the coöperation of the chemical mechanism represented by the suprarenal glands.

And it is clear that whether the rise of pressure be brought about by direct stimulation of the efferent splanchnic fibers, by asphyxia or by stimulation of afferent nerves, he had no other conception of this coöperation than that the nervous action on the cardiovascular system is accompanied by a nervous action on the adrenals which causes an increased outpouring of epinephrin.

Nobody doubts that when the peripheral end of the cut splanchnic is stimulated the liberation of epinephrin from the corresponding adrenal, which has been reduced by the nerve section from the normal spontaneous rate to zero, or at least greatly diminished, is augmented. Von Anrep is therefore entitled to conclude that in this case a part of the characteristic vascular and cardiac reactions is due to the abrupt increase in the epinephrin content of the blood. But it is one thing to use the well established fact that stimulation of the peripheral end of the splanchnic increases the output of epinephrin to explain a reaction of the denervated heart which can be shown to depend upon the secretion of epinephrin by the adrenals, and quite another thing to deduce from the occurrence of these reactions when afferent nerves are stimulated the conclusion that they must be due to an *augmented* output of epinephrin, merely because they cannot be obtained in the absence of the adrenals.² This conclusion could only be accepted if it were shown that the increase in the rate of blood flow through the coronary circulation associated with the rise of blood pressure is inadequate to produce the reactions. The increased blood flow must necessarily be accompanied by an increased supply of epinephrin to the heart vessels per unit of time, even if no increase has occurred in the rate of liberation of epinephrin from the adrenals, and the denervated heart, an exceedingly delicate test object for epinephrin, according to von Anrep, may be expected to respond just as if the epinephrin output had been increased without any material change in the rate of flow. The possibility is also

² In our experience, in the cat (von Anrep worked with dogs) a not inconsiderable acceleration of the denervated heart can usually be obtained by stimulating the central end of the sciatic or the peripheral end of the splanchnic, even when the adrenal veins are clipped. There is nothing strange about this. It is obviously dependent upon the better blood flow through the coronary vessels. Guthrie and Pike (9) showed that in the perfused mammalian heart the rate could be increased decidedly by increasing the pressure of the perfusion fluid.

present, of course, in the case of the heart as in the case of the vascular reactions, that the concentration of epinephrin may be increased by stimulation of the central end of a peripheral nerve without any increase in the rate of the discharge per unit of time.

The question of the physiological value attributed by von Anrep to the reactions of epinephrin which he has so carefully studied, especially the question of the physiological value of the heart reactions is unaffected by substituting for the supposed reflex augmentation of the output of epinephrin an automatic redistribution of the blood which, without any material change in the rate of output, carries with it an increased supply of epinephrin to organs not involved in the vasoconstriction associated with the rise of blood pressure. We suggest that in such redistributions of blood containing the epinephrin secreted by the adrenals at a relatively stable and constant rate, rather than in a sudden outpouring of epinephrin, is to be sought the mechanism of any physiological effects of this type exerted by the naturally secreted epinephrin on the organism.

To return to the question of the relation of the adrenals to *piqûre* hyperglycemia, Kahn and Starkenstein (11) availed themselves in some experiments of the now well established fact that a certain proportion of rabbits deprived of both adrenals survive for a long time or indefinitely, in good health. In our experience the proportion is something like 20 per cent. If the animals which survive some weeks be included, it is greater. The statement of Freund and Marchand (12) that after complete extirpation of both adrenals rabbits die without exception in a short time, and usually on the first day, must be based on some error of operative technique. Unfortunately Kahn and Starkenstein contented themselves with tests, mostly qualitative, for sugar in the urine. They made no quantitative estimations of the blood sugar which in an investigation of this sort cannot be satisfactorily replaced by the qualitative tests on the aqueous humor employed by Kahn. Also in some of the very few relevant protocols published by them it is seen that practically no urine was secreted after the *piqûre*, and a negative sugar test in such cases would of course possess no value. It cannot therefore be admitted that the negative results of *piqûre* obtained by these writers on rabbits deprived of the adrenals have by any means settled the question of the indispensability of the epinephrin secretion for *piqûre* hyperglycemia.

The experiments of Biberfeld (13) are even less convincing and he admits that he does not now think that observations on sugar in the

urine, the only observations he made, are satisfactory in the absence of blood sugar estimations.

The investigations of Jarisch (14) are free from this objection but are nevertheless open to criticism for other reasons. He endeavored to show that in rabbits after complete section of all the possible nervous paths from the central nervous system to the liver, piqûre was still followed by hyperglycemia when nervous connections of one adrenal were left whereas it did not cause hyperglycemia when the innervation of the adrenals was completely severed, that of the liver being intact. He estimated the blood sugar by Bertrand's method, precipitating the proteins by Schenck's method. Unfortunately he contented himself with a single blood sample, taken some time after piqûre. He compared the sugar content of this sample with a theoretical normal level and not, as we have invariably done, with that of a preliminary sample taken from the same animal. This renders the classification of some of the results as hyperglycemia quite arbitrary. In one series of ten experiments, for example, in which the innervation of the left adrenal was preserved while the remaining splanchnic distribution was severed, he counts a blood sugar content of 0.164 per cent among the hyperglycemias, very likely quite correctly. This is the series of experiments designed to show that augmented epinephrin secretion can cause hyperglycemia without the intervention of the hepatic nerves. In another series of five experiments in which the right adrenal was extirpated and all the nerves of the left adrenal cut, he classifies a blood sugar content of 0.167 per cent not as a hyperglycemia but as at "the upper limits of the normal content." This series was designed to show that in the absence of epinephrin secretion by the adrenals piqûre does not cause hyperglycemia. It must further be objected that in very few of Jarisch's experiments was the interval after the primary operation sufficiently long to permit a great accumulation of glycogen in the liver, even although the animals received cane sugar by stomach tube some time before the piqûre was made. The proof of this is the very low glycogen content of the liver in most of the experiments, even making allowance for the loss of glycogen in successful piqûre observations. For example, in three experiments in which he states that there was no hyperglycemia and in which therefore the glycogen content of the liver at the end of the experiment probably did not differ much from the content just before the piqûre, the glycogen only amounted to 0.7, 1.1 and 1.3 per cent, respectively. In five of the experiments with hyperglycemia in which the liver glycogen was estimated at the end, the per-

centages were 0, 0.2, 0.3, 0.8 and 2.1. With a good initial content of glycogen in our experiments the residual content after hyperglycemia produced by piqûre and later on by asphyxia was much greater, although the animals were not killed for a considerably longer time after the piqûre than appears to have been the case in the experiments of Jarisch.

The series of five experiments which are supposed to prove the inefficiency of the piqûre when the adrenals have been denervated although the liver nerves are intact is, we believe, completely vitiated on account of the low initial glycogen content. The percentages at the end of the experiments were 0, 0.5, 0.8, 0.9, 1.4. If there was no hyperglycemia, as Jarisch concludes, these percentages are probably not very different from the percentages before piqûre. It is vain to argue on the basis of one or two control experiments that with 1 per cent of glycogen in the liver piqûre will normally succeed. In our experience there is considerable variability in this matter in normal rabbits and there does not seem to be any reason why the production of a distinct piqûre hyperglycemia should depend solely upon the percentage of glycogen in the liver. At least one other factor, the rate of consumption of sugar in the animal, is obviously concerned. Even as regards the rate of mobilization of the glycogen it is improbable that the percentage of this substance in the liver should be the sole determining factor, and it cannot be assumed that every animal will respond in the same way to a given procedure when the glycogen store reaches a given level. The only way to be sure that a negative result is not due to too small a glycogen store is to work with animals whose livers are well filled with glycogen, and then always to make a glycogen estimation. It is impossible to decide beforehand that this or that animal will have enough glycogen to render a positive result certain after piqûre, particularly when the animal has been recently subjected to a major operation. It may also be pointed out that positive results are much more important than negative ones in a question of this kind, and that only a decided hyperglycemia should be accepted as a positive result. Negative results in animals whose glycogen content is low ought not to be used at all.

A further criticism of these experiments is that it is surely difficult to be certain that the whole nerve supply of the liver has been divided by such an operation as that practiced by Jarisch. Finally, he made the piqûre under ether anesthesia. Even if the anesthetic was administered only during the operation, how is one to be certain that a subsequent hyperglycemia was not due to the anesthesia rather than to

the piqûre? We have already shown (15) that in cats a brief administration of ether is capable of causing distinct hyperglycemia after the secretion of epinephrin has been abolished.

Among recent workers who have denied the importance of the adrenals in puncture hyperglycemia or glycosuria may be mentioned Wertheimer and Battez, Freund and Marchand and Trendelenburg and Fleischhauer. Wertheimer and Battez (16) found glycosuria in three cats following piqûre after removal of both adrenals. The experiments, however, were necessarily acute. In one case the animal was anesthetized with chloroform and, as the authors point out, it was impossible to discriminate between the effect of the anesthetic and the effect of the piqûre. In the other two cats spinal anesthesia (cocain) was employed. In five cats no definite glycosuria could be demonstrated. Blood sugar estimations were not made.

Freund and Marchand (17) conclude that the influence of piqûre is exerted directly on the liver and not through the adrenals. However, although they made numerous experiments on rabbits, none of them are entirely satisfactory. All were acute experiments, the piqûre being performed two or three hours after the removal of the adrenals, and it is difficult if not impossible in many cases to disentangle any effect of piqûre on the blood sugar from the effects of the operation and the anesthetic. The blood sugar was estimated by Bang's micro-method.

Trendelenburg and Fleischhauer (18) reached the conclusion that puncture glycosuria is not due to a "hormone" action of adrenalin discharged from the adrenals, since the rate of the discharge is not increased by the puncture. This result, however, is not arrived at by direct assay of the epinephrin coming from the adrenals, but is deduced from the fact that sugar puncture does not cause, in an anesthetized animal, a rise of blood pressure, whereas the minimal quantity of adrenalin which must be injected into a vein in order to elicit adrenalin glycosuria causes a distinct increase of blood pressure. Jarisch (14) has criticised, justly in our opinion, the deductions of Trendelenburg and Fleischhauer. For one thing, they relied entirely upon testing for sugar in the urine and did not estimate the blood sugar, the really important point. Their main argument is based upon premises by no means beyond question, and we believe that although their conclusion—that it is not an augmented epinephrin secretion which is responsible for puncture glycosuria—is correct, this cannot be established by such experiments.

Our own experiments were made on rabbits. The adrenals were removed at separate times. The interval between the first and second operation varied from eleven days to eight months. The piqûre was made ten days to eighty-one days after removal of the second adrenal. The floor of the fourth ventricle was exposed according to Eckhard's method, under local anesthesia by ethyl chlorid. A sample of blood was taken, usually from an ear-vein, before piqûre; a second sample about one to one and one-half hours after piqûre. About an hour later a third sample was drawn in order that some idea of the duration of the hyperglycemia and the maximum blood sugar content attained might be gotten. Finally asphyxia was produced by covering the mouth and nose at intervals for a period of fifteen to twenty minutes. The effect of the asphyxia on the heart-beat was sedulously controlled and a few free respirations allowed as soon as the heart was distinctly slowed, so that the asphyxia was never pushed to the point where life was in danger. A blood specimen was then drawn. The object of the asphyxia was to test whether, in the event of the piqûre yielding a negative result, a decided hyperglycemia was capable of being produced in any particular animal. We have already shown (15) that in cats with one adrenal removed and the secretion of epinephrin from the other abolished by section of its nerves, asphyxia invariably causes hyperglycemia when the glycogen content of the liver is not deficient. The animal was then killed and the glycogen content of the liver estimated by Pflüger's method, the sugar after hydrolysis of the glycogen being determined by Bertrand's method. The blood sugar was estimated by the method of Lewis and Benedict. Pearce's autoclave modification and the graduated test-tubes used by us in our previous work (1) were employed. It was recognized, of course, that when hyperglycemia had been produced by any of the procedures employed, the glycogen content as determined at the end of the experiment must be materially less than would have been found had the animal been killed at once. Accordingly before undertaking the piqûre experiments, we made a series of observations on the glycogen content of the liver under various diets, in cats whose epinephrin output had been interfered with by the operation mentioned and in rabbits and rats which had survived the removal of both adrenals. These experiments will be given in the second part of the paper.

The following condensed protocols illustrate the effects of piqûre and asphyxia on the blood sugar content in rabbits deprived of the adrenals.

Protocol. Rabbit 155, male

February 15, 1917. Left adrenal excised.

June 8, 1917. Weight, 2.625 kgm.

September 13, 1917. Right adrenal excised. From this time fed daily with carrots in addition to the ordinary diet (oats and hay daily, a carrot or a small quantity of green food once a week).

November 2, 1917. Weight. 3.275 kgm.

11.30 a.m. Obtained from ear normal blood specimen. It contained 0.102 per cent dextrose.

11.45 a.m. Piqûre.

12 30 p.m. Blood specimen from ear contained 0.205 per cent dextrose.

1.50 p.m. Blood specimen from ear contained 0.134 per cent dextrose. Asphyxia for 25 minutes, then at

2.20 p.m. Obtained blood from external saphenous vein, containing 0.216 per cent dextrose.

2.20 p.m. Killed by heart-stab, removed liver. Glycogen in liver, 7.40 per cent.

Autopsy. Accessory adrenals not found. First piqûre a little above calamus and slightly to left of mid line; second piqûre about 10 mm. below the opening of the iter in mid line.

Protocol. Rabbit 181, male

November 19, 1917. Right adrenal excised.

November 30, 1917. Left adrenal excised. Weight 2.66 kgm. Some cane sugar was added to the drinking water from this date on. Otherwise, the ordinary diet.

February 19, 1918. Weight, 2.48 kgm.

12.30 p.m. Normal blood specimen from ear contained 0.119 per cent dextrose.

12.50 p.m. Piqûre.

2.40 p.m. Blood from external jugular vein contained 0.349 per cent dextrose.

4.00 p.m. Blood from external jugular vein contained 0.449 per cent dextrose. Now caused asphyxia for 20 minutes and at

4.30 p.m. Blood obtained by cutting neck vessels, contained 0.517 per cent dextrose.

Liver at once excised; it contained 2.44 per cent glycogen. Taking the weight of the liver as 60 grams and the weight of the blood as 200 grams, the amount of glycogen which must have been mobilized merely to raise the blood sugar content to 0.52 per cent would correspond to 1.3 per cent of the liver weight. Therefore the initial content of glycogen must have been at least 4 per cent and no doubt considerably more.

Autopsy. Accessory adrenals not found. The piqûre was about 6 mm. above the calamus in the mid line.

Protocol. Rabbit 188, female

November 19, 1917. Excised right adrenal.

November 26, 1917. Gave birth to five young.

February 13, 1918. Excised left adrenal. Weight, 2.2 kgm.

Diet, cane sugar in drinking water and carrots given daily for four weeks prior to experiment, in addition to the ordinary diet.

March 12, 1918. Weight, 2.12 kgm.

10.40 a.m. Normal specimen from the ear contained 0.102 per cent dextrose. :

11.00 a.m. Piqûre.

12.10 p.m. Blood from external jugular vein contained 0.161 per cent dextrose.

Voided urine. Test with Fehling negative.

12.55 p.m. Blood from external jugular vein contained 0.176 per cent dextrose.

Now caused asphyxia for twenty minutes.

1.15 p.m. Blood from jugular vein contained 0.262 per cent dextrose. Liver at once excised. It contained 2.35 per cent glycogen.

Autopsy. Piqûre 4 mm. above calamus in mid line. No accessory adrenals found in abdomen.

In these animals a decided hyperglycemia was caused by piqûre. The same is true of asphyxia following the piqûre. In the first experiment the piqûre hyperglycemia had distinctly diminished before asphyxia was induced, and after asphyxia the blood sugar content rose to fully the maximum level obtained in the first specimen after piqûre. In the second experiment, the second blood specimen taken after piqûre contained 0.1 per cent more dextrose than the first piqûre specimen. In spite of the high grade of the piqûre hyperglycemia, the blood sugar content increased still further during asphyxia. In the third experiment where the hyperglycemia after piqûre, although quite distinct, was not so great as in the second, the specimen drawn after asphyxia also showed a decided increase in the blood sugar as compared with the second specimen after piqûre. There can be no question, then, that piqûre, like asphyxia, is capable of causing hyperglycemia in rabbits after removal of the adrenals. Obviously, as already pointed out, in a question of this kind positive results are much more important than negative ones. In the three animals the liver was well filled with glycogen, a considerable period having elapsed since the last operation.

Negative results in animals taken at too short an interval after the adrenal operation and containing little glycogen in the liver, have of course no weight at all. Not a few of the observers who have denied the possibility of producing hyperglycemia by piqûre after adrenalectomy have been misled by want of attention to this point. The following protocols are samples of our negative experiments on adrenalectomized rabbits:

Protocol. Rabbit 156, male

March 20, 1917. Left adrenal excised.

June 8, 1917. Weight, 1,775 kgm.

September 15, 1917. Right adrenal excised. From this time on the animal was fed regularly with carrots in addition to the ordinary diet.

November 13, 1917. Weight, 2.45 kgm.

11.00 a.m. Normal blood specimen from ear contained 0.101 per cent dextrose.

11.35 a.m. Piqûre. Two stabs.

12.15 p.m. Blood from cut external saphenous vein contained 0.107 per cent dextrose.

1.10 p.m. Blood from femoral vein contained 0.097 per cent dextrose. Now caused asphyxia for twenty minutes and at

1.40 p.m. Obtained blood specimen from external jugular vein, containing 0.121 per cent dextrose.

Immediately killed and excised liver, which contained 1.02 per cent of glycogen.

Autopsy.—Accessory adrenals not found. The first piqûre was 3 to 4 mm. above the calamus in the mid line, the second 6 to 7 mm. above the first in the mid line.

Protocol. Rabbit, 183, male. Weight, 2.235 kgm.

December 7, 1917. Right adrenal excised. Weight, 2.24 kgm.

Feb. 11, 1918. Left adrenal excised. Weight, 2.20 kgm. Kept on ordinary diet from this date, except that some cane sugar was given in the drinking water one day before the piqûre experiment. The weather was very cold at this time.

February 21, 1918. Weight, 2.235 kgm.

11.00 a.m. Normal blood specimen from ear contained 0.114 per cent dextrose.

11.10 a.m. Piqûre.

12.10 p.m. Blood specimen from ear contained 0.131 per cent dextrose.

1.20 p.m. Blood from ear contained 0.121 per cent dextrose. Asphyxia was now caused for twenty-five minutes, then at

1.50 p.m. Obtained blood specimen from external jugular, containing 0.128 per cent dextrose.

The neck vessels were now severed and a blood specimen obtained which contained 0.126 per cent dextrose. The liver was at once excised; its glycogen content was 2.32 per cent.

Autopsy. A small accessory adrenal was found under the left renal vein. The piqûre was 6 to 7 mm. above the calamus in the mid line.

It would not be profitable to speculate on the reason for the absence of piqûre hyperglycemia in these animals. Since precisely similar negative results may be obtained in normal animals, we do not see how they can be connected with the presence or absence of the adrenals. While everybody is agreed that a high glycogen content is favorable for the occurrence of piqûre hyperglycemia, and that a very low glycogen content is incompatible with it, there is no evidence as already

stated, that hyperglycemia must necessarily be obtained in an animal with more than a certain percentage of glycogen in its liver, whereas in animals with less than that percentage it can never be obtained.

The following protocols illustrate the results on normal rabbits with the same technique as that employed for the adrenalectomized animals:

Protocol. Rabbit 186

Male, which had been long in stock. Weight, 1.80 kgm. Ordinary diet.

February 27, 1918.

11.00 a.m. Normal blood specimen from ear contained 0.107 per cent dextrose.

11.15 a.m. Piqûre.

12.15 p.m. Blood from ear contained 0.374 per cent dextrose.

1.20 p.m. Blood from ear contained 0.46 per cent dextrose. Asphyxia was now caused for twenty-five minutes and at

1.45 p.m. A blood specimen was obtained with 0.514 per cent dextrose.

Ten minutes later the blood vessels in the neck were severed and another blood specimen obtained with 0.52 per cent dextrose. The liver was immediately excised. Its glycogen content was 2.96 per cent. The liver weighed 61 grams. Taking the total blood as 140 grams, the amount of glycogen which must have been mobilized merely to raise the blood sugar to 0.52 per cent would represent almost 1 per cent of the liver weight. The glycogen content before piqûre must therefore have been at least 4 per cent and no doubt was considerably more.

Autopsy. The piqûre was 5 mm. above the calamus in the mid line.

Protocol. Rabbit 182

Normal female from the stock. (Control for rabbit 181, and on the same diet).

February 19, 1918. Weight, 2.63 kgm.

12.10 p.m. Normal blood specimen from ear contained 0.11 per cent dextrose.

12.30 p.m. Piqûre.

1.40 p.m. Blood from ear contained 0.367 per cent dextrose.

3.00 p.m. Blood from ear contained 0.308 per cent dextrose. Asphyxia was now caused for twenty minutes and at

3.20 p.m. A blood specimen obtained from the ear contained 0.367 per cent dextrose.

The animal was now bled from the throat and a specimen obtained at 3.30 p.m. with 0.417 per cent dextrose. The glycogen content of the liver was 2.04 per cent. If only the amount of glycogen which must have been mobilized to make up a blood sugar content of 0.42 per cent be added, the liver must have contained before the piqûre experiment at least 3 per cent of glycogen and doubtless it contained more.

Autopsy. The piqûre was 4 to 5 mm. above the calamus in the mid line.

The following two protocols illustrate the negative results of piqûre obtained on normal rabbits just as on adrenalectomized rabbits..

Protocol. Rabbit 187, male

March 11, 1917. Weight, 1.41 kgm. No food given (except water) for two days prior to experiment.

10.00 a.m. Normal specimen of blood from ear contained 0.124 per cent dextrose.

10.15 a.m. Piqûre.

11.15 a.m. Blood from external jugular vein contained 0.136 per cent dextrose.

12.15 p.m. Blood from external jugular vein contained 0.130 per cent dextrose.

Now caused asphyxia for twenty minutes.

12.35 p.m. Blood from external jugular vein contained 0.142 per cent dextrose.

Severed blood vessels in neck and bled to death. A specimen of this mixed blood contained 0.151 per cent dextrose. The liver was at once excised; it contained 0.34 per cent glycogen.

Autopsy. The piqûre was 6 mm. above the calamus in the mid line.

Protocol. Rabbit 157

Normal female from stock. It had carrots regularly in addition to the ordinary diet for two weeks before the piqûre experiment.

November 13, 1917. Weight, 2.0 kgm.

11.15 a.m. Normal blood specimen from ear contained 0.122 per cent dextrose.

11.50 a.m. Exposed and opened the occipito-atlantoid membrane under local ethyl chlorid anesthesia, as in all the other experiments; but did not perform piqûre as yet.

12.40 p.m. Blood specimen from ear contained 0.116 per cent dextrose.

12.55 p.m. Piqûre made (two stabs).

2.10 p.m. Blood from femoral vein contained 0.10 per cent dextrose. Asphyxia was now induced for twenty minutes and at

2.30 p.m. A blood specimen was obtained from the external jugular vein with 0.101 per cent dextrose.

The animal was at once killed by heart stab. The liver contained only a trace (less than 0.05 per cent) of glycogen.

Autopsy. First piqûre, 3 to 5 mm. above the calamus in the mid line, second piqûre, 7 to 8 mm. above the first and a little to the left of the mid line.

The object of the experiment just cited was to control the effect of the operation as such, apart from the piqûre, on the blood sugar. The experiment failed for this purpose because of the low glycogen content of the liver (in spite of the carrot diet) which did not permit either the subsequent piqûre or asphyxia to cause any hyperglycemia. The following protocol gives the data of an experiment of the same kind, but on a rabbit whose liver was well filled with glycogen. It will be seen that the operation as such, with any attendant emotional excitement, caused no hyperglycemia.

Protocol. Rabbit 189, male

Diet. Sugar in drinking water and carrots for five days prior to the experiment, in addition to the ordinary diet. The animal took the sugared water readily. Weight 2.0 kgm.

9.55 a.m. Normal blood specimen from ear vein contained 0.126 per cent dextrose.

10.15 a.m. Under local anesthesia (ethyl chlorid) exposed floor of fourth ventricle but did not perform piqûre as yet. Sutured the wound.

11.20 a.m. Blood specimen from ear vein contained 0.124 per cent dextrose.

11.30 a.m. Piqûre performed.

12.30 p.m. Blood specimen from ear vein contained 0.249 per cent dextrose. Asphyxia was now caused for twenty minutes and at

1.00 p.m. A blood specimen was obtained from the external jugular vein. It contained 0.343 per cent dextrose. Urine voided at this time gave a marked reduction with Fehling's solution. The animal was killed by bleeding from the neck vessels. The liver, excised immediately, weighed 82 grams and contained 7.14 per cent of glycogen.

Autopsy. The piqûre was 4 mm. above the calamus in the mid line.

The results of a number of preliminary experiments on normal rabbits in which glycogen determinations were not made, are given in table 1, in order to emphasize the point that a negative piqûre experiment in an adrenalectomized animal must not be attributed off hand to the absence of the adrenals. The six rabbits had lived a long time in the laboratory under identical conditions and on the same (ordinary) diet. Carrots were added one day before the experiment. It will be seen that three of the animals yielded distinctly positive results with piqûre. In two the result was negative, in one doubtful.

TABLE 1
Percentage of blood-sugar

NORMAL	PIQÛRE	ASPHYXIA
0.135	0.162	0.163
0.125	0.390	
0.130	0.134	
0.110	0.263	
0.101	0.178	
0.102	0.115	0.122

TABLE 2
Adrenalectomized rabbits

DATE OF GLYCOGEN ESTIMATION	NUM- BER OF ANIMAL	ADRENALS EXCISED		PERCENT- AGE OF GLYCO- GEN IN LIVER	REMARKS
		First	Second		
1917 October 23	145	1917 January 18	1917 September 17	6.42	Carrots daily since last operation in addition to usual diet*
November 2	155	February 15	September 13	7.40	Same as for 145
November 13	156	March 20	September 15	1.00	Same as for 145
		1916			
November 5	158	December 7	September 22	0.68	Attempted piqûre. Died; liver taken after one-half hour
1918 February 19	181	1917 November 19	November 30	2.44	Cane sugar daily since last opera- tion, in addition to ordinary diet
			1918		
February 21	183	December 17	February 11	2.32	Cane sugar one day before experi- ment (ordinary diet). Very cold weather
February 25	184	December 7	February 13	Trace	Ordinary diet. Rabbit has mange, not eating well, losing weight
March 12	188	November 19	February 13	2.35	Cane sugar and car- rots daily since last operation, in addition to ordi- nary diet

* Rabbit 145 was sacrificed for glycogen estimation. In all the others a piqûre experiment was done before the liver was excised. The ordinary diet for rabbits consisted of oats and hay daily, with a carrot or a small piece of green food once a week.

PART II. RELATION OF THE ADRENALS TO THE GLYCOGEN CONTENT OF THE LIVER

Several of the writers on the problem of the possibility of producing experimental glycosurias and hyperglycemias after removal of the adrenals have raised the question whether the negative result might not be due to inability of the liver to form or to store glycogen in the adrenalectomized animal. Since as shown above the result is not negative, this question does not arise. Nevertheless, before making our observations on piqûre and in the course of them, we made a con-

TABLE 3
Normal control rabbits

DATE	NUM- BER OF ANIMAL	GLYCOGEN PERCENT- AGE IN LIVER	REMARKS
<i>1917</i>			
October 23	144	2.80	Ordinary diet
October 29	150	4.73	Ordinary diet with addition of carrots for 6 days prior to experiment
November 22	160	2.58	Ordinary diet with addition of carrots for one week prior to experiment
November 13	157	Trace*	Ordinary diet with addition of carrots for one week prior to experiment. (Piqûre, etc.)
<i>1918</i>			
February 19	182	2.04	Cane sugar daily in addition to ordinary diet since November 30, 1917 (Piqûre, etc.)
February 27	185	1.74	Ordinary diet. Piqûre attempted. Died
February 27	186	2.96	Ordinary diet. (Piqûre, etc.)
March 11	187	0.34	Starved two days. Piqûre (negative)

* Less than 0.05 per cent.

siderable number of experiments on cats, rabbits and rats, in order to obtain information upon the glycogen content of the liver in animals in which the adrenals had been removed or, in the case of the cats, the secretion of epinephrin interfered with. The rabbits used had survived the removal of the second adrenal eleven days to nine months, when killed for the glycogen determination. Details as to diet, glycogen content, etc., are given in table 2, which includes a certain number of animals in which the glycogen was determined after hyperglycemia had been produced by piqûre and by asphyxia (see protocols in part I.). The glycogen, estimated at the end of these experiments must, of course, be less than the actual content before the piqûre.

In table 3 are displayed the results of a number of glycogen determinations on normal control rabbits. It will be seen that there is no material difference between the results in table 2 and table 3.

Since cats do not survive the removal of both adrenals, we excised one adrenal and divided the nerves of the other so as to abolish or reduce greatly the output of epinephrin. Protocols of two such experiments follow:

Protocol. Cat 125, male

Diet. Liver, milk, occasionally fish and a daily ration of rice or potatoes boiled with milk.

August 18, 1917. Weight, 2.64 kgm. Excised right adrenal. It weighed 0.266 gram and contained 0.12 mgm. epinephrin. Extirpated left semilunar ganglion. Excised left superior cervical ganglion.

September 18, 1917. Weight, 1.92 kgm. Left pupil contracted and nictitating forward.

11.25 a.m. Urethane 3 grams (stomach).

2.50 to 3.15 p.m. Inserted tracheal and jugular cannulae; artificial respiration; coeliac and mesenteric arteries tied and a lobe of liver at once excised for glycogen estimation. Completed cava pocket in usual manner.

3.15 p.m. Both pupils equal and nictitating membranes slightly forward.

3.20 p.m. Pocket experiment two minutes. No eye reactions.

3.24 p.m. Pocket experiment five minutes. No eye reactions.

3.30 p.m. Pocket experiment nine minutes. No eye reactions.

3.44 p.m. 0.5 cc. 1:1,300,000 adrenalin. Very good pupil dilatation in 11.2 seconds. No nictitating reaction.

3.48 p.m. 0.5 cc. 1:2,600,000 adrenalin injected. Good pupil reaction in 11.4 seconds. No nictitating reaction.

3.53 p.m. 0.5 cc. 1:4,000,000 adrenalin injected: Small pupil reaction in 13.8 seconds. No nictitating reaction.

3.58 p.m. 0.5 cc. 1:5,200,000 adrenalin injected. Small pupil reaction in 13.6 seconds. No nictitating reaction.

Now collected the following blood samples from the adrenal (short pocket):

Sample 1, 1.4 grams in one minute (1.4 grams per minute).

Sample 2, 4.4 grams in four minutes (1.1 grams per minute).

Sample 3, 3.8 grams in eight minutes (0.475 gram per minute).

Obtained blood from abdominal aorta. Left adrenal weighed 0.257 gram, and contained 0.17 mgm. epinephrin. Glycogen in lobe of liver removed at beginning of experiment, 4.26 per cent.

The epinephrin assay of the adrenal vein blood gave the following results:

The tests with rabbit intestine and uterus segments showed that even the third adrenal specimen of blood could not have contained more than 1:260,000,000 epinephrin, corresponding to an output of

0.0000018 mgm. per minute for the animal or 0.0000007 mgm. per minute per kilogram of body weight; i.e., not more than one-three hundred and fiftieth of the normal output as estimated by the segments.

The second adrenal specimen did not give a much greater effect than the indifferent arterial blood and a far smaller effect than indifferent blood containing 1:260,000,000 adrenalin.

The eye reactions were negative even when blood was collected in the cava pocket for nine minutes. Yet distinct reactions were obtained when 0.5 cc. of a 1:5,300,000 solution of adrenalin was injected. Accordingly, as tested in this way, the output could not have amounted to 0.00001 mgm. per minute for the animal or 0.000004 mgm. per kilogram of body weight per minute; i.e., not one-one hundred and fiftieth of the normal, as estimated by eye reactions.

Protocol. Cat 113, male

Same diet as for cat 125.

July 20, 1917. Weight, 2.725 kgm. Excised right adrenal. It weighed 0.152 gram and contained 0.16 mgm. epinephrin. Extirpated left semilunar ganglion and severed lumbar chain below the diaphragm.

August 16, 1917. Weight, 2.545 kgm. Right splanchnics divided.

August 30, 1917. Weight, 2.71 kgm. Blood sugar tests made as follows: Normal specimen, 0.089 per cent; specimen collected after frightening, 0.084 per cent; specimen collected after asphyxia, 0.153 per cent.³

August 31, 1917. Excised left superior cervical ganglion.

September 18, 1917. Weight, 2.41 kgm. Condition excellent.

10.15 a.m. 5 grams urethane (stomach).

11.50 a.m. Inserted tracheal and jugular cannulae; tied coeliac and mesenteric arteries; at once clamped off and removed the left and part of the middle lobe of the liver for glycogen estimation. Then completed a cava pocket in the usual way.

12.20 p.m. Left pupil wider than right; both nictitating slightly forward.

12.20 p.m. Pocket experiment two minutes. Small pupil and nictitating reactions in 10 seconds.

12.24 p.m. Pocket experiment, one minute. Small pupil and nictitating reactions in 13.2 seconds.

12.26 p.m. Pocket experiment, three minutes. Small pupil and slight nictitating reactions in 11 seconds. (Not much different from observation at 12.20.)

12.34 p.m. 0.5 cc. 1:1,300,000 adrenalin injected. Very large pupil and nictitating reactions in 6.2 seconds.

12.40 p.m. 0.5 cc. 1:2,600,000 adrenalin injected. Very large pupil and nictitating reactions.

³ The blood sugar results of this experiment have been already cited in our paper in this Journal, 1917, xliv, 543.

- 12.41 p.m. 0.5 cc. 1:2,600,000 adrenalin injected. Same result.
12.46 p.m. 0.5 cc. 1:2,000,000 adrenalin injected. Very good pupil reaction in 6 seconds. (Nictitating still back.)
12.48 p.m. 0.5 cc. 1:2,600,000 adrenalin injected. Good pupil reaction in 8.2 seconds.
12.50 p.m. 0.5 cc. 1:4,000,000 adrenalin injected. Probably slightly larger reaction than that produced by blood collected for two to three minutes in the cava pocket.

Now collected blood specimens from adrenal (short pocket) as follows:

Sample 1, 1.2 grams in 35 seconds (2 grams per minute).

Sample 2, 7.0 grams in four and one-half minutes (1.55 grams per minute).

Sample 3, 9.0 grams in nine minutes (1 gram per minute).

Collected blood from jugular vein and also from abdominal aorta. Left adrenal weighed 0.141 gram and contained 0.15 mgm. epinephrin. Glycogen in liver removed at beginning of experiment, 4.75 per cent.

The eye reactions in this animal indicated that the output of epinephrin could not have been more than 0.00004 mgm. per minute for the animal or 0.000017 mgm. per kilogram of body weight per minute; i.e., not more than one-thirty-fifth of the normal output, as estimated by eye reactions. The rabbit uterus and intestine segment tests showed that the third adrenal specimen contained about 1:65,000,000 epinephrin, corresponding to an output of 0.000015 mgm. per minute for the animal or 0.000006 mgm. per kilogram of body weight per minute; i.e., one-fortieth of the normal as estimated by the segments.

The glycogen content of the liver in these two cats was 4.26 and 4.75 per cent, respectively. In two normal control cats, in which the operative procedure followed in cats 125 and 113 for obtaining a lobe of the liver was imitated under urethane, with ligation of the coeliac and mesenteric arteries, the glycogen content of the liver was 1.95 and 2.55 per cent respectively. In a third normal cat, killed instantaneously without urethane, the content was 4.13 per cent.

Since, as is known, a large proportion of rats survive the excision of both adrenals we made some observations on these animals also. It has been stated by Schwarz (19) that rats do not survive more than a day if both adrenals are removed at one time. He therefore left an interval between the two operations. He is certainly mistaken in this matter for we excised the two adrenals at one time in thirteen rats. Of these eight died in one to fourteen days. The remaining five recovered completely and were sacrificed for the glycogen estimation. The results are given in table 4. In table 5 are given for comparison the glycogen percentages found in five normal rats and in three rats on which a laparotomy had been performed in order to control approxi-

TABLE 4

Adrenalectomized rats

DATE OF GLYCOGEN ESTIMATION	NUMBER OF ANIMAL	DATE OF ADRENALECTOMY	GLYCOGEN PERCENTAGE IN LIVER	REMARKS
<i>1917</i>		<i>1917</i>		
November 13	159	October 28	5.01	Ordinary diet*
November 22	162	October 28	2.41	Ordinary diet
December 14	165	December 3	Trace	Unleavened bread and milk; lost weight; accessory adrenal found
December 24	167	December 11	1.40	Ordinary diet (no milk for two days before experiment). Small accessory adrenal found
<i>1918</i>				
January 4	169	December 11	2.99	Unleavened bread, water, no milk; butter for four days

* The ordinary diet for rats consisted of bread, corn, oats and milk daily and a small piece of cabbage once a week.

TABLE 5

Normal Rats

DATE OF GLYCOGEN ESTIMATION	NUMBER OF ANIMAL	GLYCOGEN PERCENTAGE IN LIVER	REMARKS
<i>1917</i>			
November 22	161	2.69	Carrots and sugar for 1 week before experiment in addition to ordinary diet
November 22	163	1.48	Ordinary diet
December 14	166	2.59	Unleavened bread and milk
December 29	168*	2.32	Ordinary diet but no milk for two days before experiment
<i>1918</i>			
January 4	170*	3.98	Unleavened bread, water but no milk (butter four days prior to experiment)
January 25	171*	4.45	Only unleavened bread and water for two weeks before experiment
January 25	172	5.19	Only unleavened bread and water for two weeks before experiment

* To control any general effects of the operation in the adrenalectomized rats, a laparotomy was performed on these three normal rats on December 19, 1917. Adrenalectomy was not performed but otherwise the operation was similar.

mately the effects, as regards trauma, anesthesia, etc., of the operation on the adrenalectomized rats but without removal of the adrenals. No essential difference is shown in the two tables.

Schwarz has stated that the livers of adrenalectomized rats after feeding with dextrose or cane sugar contained considerable quantities of glycogen. His protocols show no definite deficiency as compared with normal rats. On the other hand, he asserts that when carbohydrate is supplied in the form of starch as in feeding "Semmeln," the livers of the adrenalectomized rats are practically free from glycogen, while normal rats with the same diet show a good content. Although the fact that with sugar feeding the adrenalectomized animals form and store considerable quantities of glycogen is sufficient to exclude the idea that any essential change in the process of glycogenesis is caused by the removal of the adrenals, we made some experiments in order to control Schwarz's observations. Two adrenalectomized rats were fed from the time of the operation with a diet certainly free from added sugar, the "matzo" (matzoh), or unleavened bread used during the Jewish Passover. Of these rats, one had a glycogen content of 2.99 per cent. The control rat (170 in table 5) had a content of 3.98 per cent. The other adrenalectomized rat had only a trace of glycogen in the liver but the animal was losing in weight, had been apathetic for some days and was sacrificed because it was feared it was going to die. The control normal rat (166, table 5) had 2.59 per cent. Two other control rats (171 and 172, table 5), were fed solely on unleavened bread and water for two weeks. The glycogen contents were 4.45 per cent and 5.19 per cent respectively.

Kahn and Starkenstein (11) made a few experiments to test the results of Schwarz. They likewise state that rats do not survive when both adrenals are removed at one sitting. Indeed, according to them, if a shorter interval than three to four weeks is left between the first and second operations, death ensues within two days after the second operation. As we have already pointed out, this conclusion is certainly erroneous. A quite considerable proportion of rats survive for a much longer time after simultaneous removal of both adrenals. Kahn and Starkenstein state that adrenalectomized rats fed on a diet of milk, "Semmeln" and some oats, do not store glycogen in the liver except in traces. The number of experiments performed by them was very small and the few protocols given in their paper do not support this conclusion. In the three adrenalectomized animals in which glycogen determinations were made, the glycogen content in one was

2.38 per cent. In another, piqûre had been done before the liver was obtained for the glycogen determination and if a hyperglycemia had been produced the glycogen content before piqûre would doubtless have been considerably higher than that actually found. They did not make any estimations of blood sugar. The interval between the last adrenal operation and the glycogen determination was in general too short for the post-operative depletion of the glycogen store to be certainly made good, especially in view of the fact that they did not purposely feed sugar to the animals. We have had positive results even on a diet containing practically no sugar. That adrenalectomized rats will sometimes show only a trace of glycogen in the liver is true enough, but this is also the case with normal rats. It is necessary before comparing so-called control animals with the operated animals to know that the consequences of the operation as such on the glycogen store have been entirely eliminated. And this can never be assumed with absolute certainty in any particular animal, especially when only a few days have elapsed since the operation. Schwarz anesthetized the rats in order to administer sugar, etc., by the stomach tube and as he fed them in this way on three successive days the effects of the anesthesia may not have been entirely negligible.

SUMMARY

1. In rabbits which have survived the removal of both adrenals and have recovered from the operation, and whose livers are well filled with glycogen, piqûre causes decided hyperglycemia just as in normal rabbits. The hypothesis that piqûre hyperglycemia is caused in the same way as the hyperglycemia produced by injecting adrenalin, by an increased liberation of epinephrin from the adrenals into the blood, must therefore be abandoned. We have previously shown (in cats) that the hyperglycemia associated with asphyxia and with ether anesthesia is likewise not dependent upon the secretion of epinephrin. In the present research we have had many opportunities to observe that hyperglycemia is caused by asphyxia in adrenalectomized rabbits.
2. The results of previous observers who have failed to obtain piqûre hyperglycemia in rabbits after extirpation of both adrenals are due to the fact that they have performed the piqûre immediately after the adrenalectomy, or if an interval has been allowed it has been too short to permit complete recovery from the adrenal operation and the liver has been insufficiently stored with glycogen. Even when a consider-

able interval has elapsed after the adrenal operation, the state of nutrition of the animal or the diet has sometimes been unfavorable for glycogen accumulation and therefore a positive result could not be expected.

3. There is no real evidence that piqûre increases the rate of liberation of epinephrin from the adrenals.

4. It is pointed out that the reactions of denervated vascular regions and of the heart, isolated from extrinsic nervous influence by section of the vagi and excision of the stellate ganglia, which have been interpreted as showing that the rate of liberation of epinephrin is increased by stimulation of afferent nerves and by asphyxia, have a different significance.

5. The formation and storing of glycogen in the liver is not affected by removal of both adrenals in rabbits, or by removal of one adrenal and section of the nerves of the other in cats with consequent abolition or marked reduction in the rate of liberation of epinephrin. In rats, also, extirpation of the adrenals produces no essential change in the capacity of the liver to form and store glycogen.

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FURTHER OBSERVATIONS ON THE RELATION OF THE ADRENALS TO CERTAIN EXPERIMENTAL HYPER- GLYCEMIAS (ETHER AND ASPHYXIA)

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The question whether the epinephrin output of the adrenals is essentially concerned in the production of certain experimental hyperglycemias has been much debated. Two main ways of approaching the question are theoretically open: *a*, the estimation of the output of epinephrin under the action of the factors inducing the experimental hyperglycemia, in order to show whether this can be sufficiently great to permit the introduction into the blood of the necessary excess of epinephrin as determined by artificial injection; *b*, study of the blood sugar content in the absence of the adrenals or after interference with their epinephrin secretion, when the conditions which cause hyperglycemia with intact adrenals are induced. The first method of approach might seem to be the more direct, but it has been little used. Such results as have been obtained on the rate of output of epinephrin in ether narcosis and asphyxia are quite unfavorable to the view that epinephrin plays any sensible rôle in the production of the hyperglycemia.

Of the numerous researches made by the second method many, unfortunately, however interesting in other relations, throw no light upon the question at issue because essential conditions were not fulfilled. The most important of these seem to be the following:

1. The glycogen store must be adequate to permit of a decided hyperglycemia. Negative results cannot otherwise be accepted. The only sure way is to estimate the liver glycogen.

2. Only animals which have completely recovered from the effects of the operation practised to eliminate the adrenal epinephrin output should be used. Cats as employed by us, after removal of one adrenal and section of the nerves of the other, and rabbits which have survived double adrenalectomy, fulfil this condition.

3. The question of the rôle of the adrenals should not in the present state of our knowledge be complicated by unnecessary interference with the nerve supply of the liver. For example, negative results after section of both splanchnics cannot be used to determine the question of the indispensability of the adrenals. In our experiments on ether, asphyxia (1) and piqure hyperglycemia (2) only one splanchnic was cut in the cats and neither splanchnic in the rabbits.

We believe it is inadvisable to complicate the question whether the adrenals are essential for the development of experimental hyperglycemias with speculations on the possible output of epinephrin from the diffuse chromaffine tissue. There is no evidence as to the possible magnitude of the output of epinephrin, if there is any output, from these scattered chromaffine cells and no obvious way of investigating the matter.¹ Our own experiments have been concerned solely with the adrenals. Whether they are essential for the hyperglycemias studied is a question which can be definitely settled. We believe it has been definitely decided in the negative, and that the so-called "adrenalin hypothesis" should be abandoned, at least in the case of these forms of hyperglycemia.

Recently, however, Keeton and Ross (3) have published a paper on the mechanism of ether hyperglycemia in dogs, in which they make the point that a short etherization is associated with some hyperglycemia even when both splanchnic nerves have been cut, but that this does not persist, under continuous insufflation of ether, as it does in the case of normal dogs. Incidentally they raise the question whether the etherization in our experiments was continued long enough. They think it was not, and that we might have obtained a different result with a longer period of etherization. For this reason they do not consider that we have demonstrated conclusively that ether hyperglycemia can occur in the absence of the epinephrin output of the adrenals. Section of both splanchnics introduces the complication that the innervation of the liver is greatly interfered with, whereas our object was to interfere only with the adrenals, and in this regard

¹ If the diffuse chromaffine tissue is under the control of nerves in respect of any output of epinephrin, as is to be supposed since it represents sympathetic nerve cells, the output could be greatly interfered with, it may be assumed, by section of sympathetic fibers, including the splanchnics, or by appropriate spinal cord section. In animals which have survived in good health some of the operations practised by us, it seems probable that a large part of the epinephrin output, if any exists, of the diffuse chromaffine tissue was suppressed as well as that of the adrenals.

our experiments are not really comparable with those of Keeton and Ross. Nevertheless, as courteous criticism is always helpful and as, of course, it might be possible that a longer period of etherization should develop a difference between normal animals and those in which the adrenal epinephrin output has been interfered with, we have made some experiments in which the blood sugar was determined at the end of a short period (20 minutes) and again at the end of a much longer period of etherization (80 to 90 minutes). The experiment was wound up with a period of asphyxia and another blood sample collected, since asphyxia is one of the surest methods of producing hyperglycemia, and in the event of a negative result with ether the asphyxia sample serves as a control to show whether the nutritive condition of the animal, especially the glycogen store, was compatible with a well marked hyperglycemia. In addition, the glycogen content of the liver was estimated.

Before proceeding to these experiments, however, it seems necessary to explain what we aimed at in our previous experiments. Keeton and Ross state that "of four of their (Stewart and Rogoff's) experiments, only two show a gain (0.087 to 0.151 and 0.142 to 0.176) that does not fall within the limit of experimental error. The other two (0.200 to 0.233 and 0.092 to 0.098) are not significant." This criticism is based on a misapprehension. We made a number of blood sugar experiments on each cat at different dates. In some instances the results were negative in one of the experiments, while on another day, perhaps a week or more thereafter, the animal meanwhile being specially fed, the results were positive, doubtless owing to the difference in the nutritional state (glycogen content of liver), a condition which is known to be of the highest importance for the production of such experimental hyperglycemias. Precisely similar results are obtained in normal animals.

In the new experiments rabbits which had survived the removal of both adrenals were employed, and here of course there was no question of any residual liberation of epinephrin from the adrenals. The animals had perfectly recovered from the primary operations, and for 8 to 11 days before the blood sugar tests were placed on a diet of carrots in addition to the routine diet of oats and hay, and sugar was added to the drinking water twice a day. The glycogen content of the liver was estimated at the end of the experiment. The results, as will be seen from the following protocols, were entirely confirmatory of our results on cats.

Condensed protocol. Rabbit 409

September 19, 1919. Excised right adrenal.

October 21, 1919. Excised left adrenal. Weight of rabbit 2.51 kgm.

November 17 to November 25, 1919. Carrots in addition to usual diet (oats and hay) and cane sugar added to drinking water, twice daily.

November 25, 1919. Condition excellent. Weight 2.67 kgm.

9.30 a.m. 2 cc. blood (from ear vein) contained 0.13 per cent dextrose.

9.40 a.m. Started light etherization.

10.05 a.m. 2 cc. blood (from ear vein) contained 0.16 per cent dextrose. Continued light etherization for an hour longer.

11.10 a.m. 2 cc. blood (from ear vein) contained 0.27 per cent dextrose. Now discontinued etherization.

11.25 a.m. Started asphyxia and continued it intermittently till

11.45 a.m. 2 cc. blood (from ear vein) contained 0.37 per cent dextrose.

The liver, excised and hydrolyzed at once, contained 3.13 per cent of glycogen. The liver weighed 40.3 grams.

Of course, the glycogen content at the beginning of the experiment would be decidedly greater than that estimated at the end.

Condensed Protocol. Rabbit 410

September 19, 1919. Excised right adrenal.

October 21, 1919. Excised left adrenal. Weight of rabbit 3.18 kgm.

November 17 to November 28, 1919. Carrots in addition to usual diet (oats and hay) and cane sugar added to drinking water, twice daily.

November 28, 1919. Condition excellent. Weight 3.25 kgm.

9.30 a.m. 2 cc. blood (from ear vein) contained 0.11 per cent dextrose.

9.40 a.m. Started etherization (to surgical anesthesia).

10.00 a.m. 2 cc. blood (from ear vein) contained 0.27 per cent dextrose. Continued etherization (lightly) for an hour longer.

11.05 a.m. 2 cc. blood (from ear vein) contained 0.41 per cent dextrose. Now discontinued etherization.

11.15 a.m. Started asphyxia: after 7 minutes of asphyxia (with a towel over nose) applied off and on (being guided by the slowing of the heart rate) the circulation became poor and the respirations shallow and slow. Artificial respiration was started and an attempt made to obtain blood from the femoral vein, but failed, owing to the poor blood flow. The chest was opened and 2 cc. of blood were obtained from the right heart. The heart was beating feebly and the blood was dark. This specimen contained 0.53 per cent dextrose.

The liver was excised 4 minutes after the last specimen was obtained and hydrolyzed at once. It contained 2.5 per cent of glycogen at the end of the experiment. At the beginning the glycogen content must have been greater. The surplus sugar in the blood at the end would alone correspond to an additional amount of 0.7 per cent glycogen in the liver.

Our results on asphyxial hyperglycemia (1), (2) both in cats after interference with the adrenal epinephrin output and in rabbits after

removal of both adrenals are quite as conclusive as those on ether hyperglycemia. Where the liver was well filled with glycogen a marked hyperglycemia was invariably obtained. What interpretation can possibly be placed upon such results except that the adrenal epinephrin is not essential to the production of these hyperglycemias?

Yet a quite recent writer, Yamakami (4), seems to think that direct evidence of this kind can be set aside because he has, as he supposes, shown that asphyxial blood, obtained from one normal rabbit and injected into another normal rabbit causes a rise in the sugar content of the blood. Even if the results which he quotes be accepted as proving a distinct augmentation in the blood sugar of the recipient rabbit, in addition to any increase due to the sugar actually present in the injected asphyxial blood (and not more than half of the experiments reproduced in his table could be considered positive), they are irrelevant to the question of the rôle of the adrenals in asphyxial hyperglycemia. They do not warrant "the hypothesis that adrenalin in the asphyxial blood is responsible because we do not know at present any other substance than adrenalin in the blood which can give rise to the enhanced sugar content." He endeavors to exclude changes in carbon dioxide content and possible changes in H-ion concentration in the asphyxial blood as factors. It would seem a more crucial test to estimate the epinephrin in the blood injected, since it is universally acknowledged that a certain amount of epinephrin will cause hyperglycemia. Underhill (5) showed that very large quantities of adrenalin introduced by continuous intravenous infusion into non-anesthetised rabbits (as much as 333 cc. of a 1:250,000 solution) did not cause glycosuria. It must be noted that the heart blood was taken from nearly dead, or in half the cases, from actually dead rabbits. With the extreme slowing of the blood flow in the inferior cava the concentration of epinephrin in the heart blood, so long as the adrenal epinephrin output was not affected, would tend to rise. The Japanese author seems to have had some suspicion that the adrenalin hypothesis might not have much to support it, for he continues "of course, we cannot venture to claim that hyperadrenalinemia was proved by our experiments to exist in asphyxia." Curiously enough he states early in the paper that "in order to solve the problem whether the adrenals are involved in the asphyxial hyperglycemia it seems to be the wisest method to study this hyperglycemia in animals whose adrenals were removed entirely," and he suggests rabbits which have survived double adrenalectomy as the most suitable. But he makes no mention of our

observations on piqûre and asphyxial hyperglycemia in such rabbits, which demonstrate that the adrenals are unnecessary. Nor does he utilize in his own observations what would be a really crucial experiment, the injection of asphyxial blood from an adrenalectomized rabbit into a normal rabbit, and into another adrenalectomized rabbit. He seems to confuse our method of collecting adrenal vein blood with the method of collecting specimens for blood sugar estimations. We did not of course anesthetize the animals, perform a laparotomy, etc., to obtain blood specimens when we were determining whether asphyxia could cause hyperglycemia in the absence of epinephrin discharge from the adrenals.

Kellaway (6) has recently verified our conclusion that the adrenal epinephrin output is not essential to the production of the hyperglycemia induced by asphyxia. He states that we have "denied that the suprarenals play any part in producing the hyperglycemia." We have not as a matter of fact put our conclusion in so absolute a form because, although our observations do not reveal any essential difference between normal animals and animals with the epinephrin output interfered with, in the degree of the hyperglycemia or the ease and certainty with which it is induced, it would be very difficult in experiments of this type to bring out clearly a small quantitative difference if such existed. Kellaway's blood sugar results in normal cats and in cats after interference with the adrenal epinephrin output are very much like our own, despite the fact that in his observations both splanchnics were divided and the innervation of the liver crippled.

His contention that increased adrenalin output is a factor in the hyperglycemia is not supported by his results so far as we can see. In most of his experiments he gets a good hyperglycemia with anoxemia (or asphyxia) after interference with the epinephrin output, exactly as we found. He has not estimated the liver glycogen in any of his animals nor does he indicate anywhere that he realizes its importance. He seems to assume that "the value of the threshold for anoxemia as regards hyperglycemia" can be fixed once for all for a given animal and that if breathing a certain percentage of oxygen causes hyperglycemia days or weeks before section of the splanchnics or removal of the adrenals, and fails to cause it after those operations, the difference must necessarily be due to the absence of epinephrin. It is impossible to accept the conclusion that because in one cat (exper. 14) "there was a very close correspondence between the production of a pupillary paradox and a rise in blood sugar" before section of the splanchnics and removal of

the adrenals, and because both reactions were absent or greatly reduced after these operations "it seems evident that in this cat the blood sugar effects were largely due to adrenalin." The failure to obtain hyperglycemia at the end of the experiment on the day after removal of both adrenals proves nothing at all, especially in the absence of any check on the glycogen store. The isolated observation (in exper. 15) that the intravenous injection of a quantity of adrenalin (0.1 cc. adrenalin 1 in 200,000) which was inadequate to elicit the paradoxical pupil reaction yet caused a hyperglycemia, is cited as further evidence that in asphyxial hyperglycemia adrenalin plays an important part. There was already a hyperglycemia present on account of the subjection of the animal to anoxemia but the apparent slight increase in blood sugar in the specimen taken after injection of the adrenalin is definitely attributed to the 0.0005 mgm. of adrenalin injected.

Kellaway's acceptance of the statements in the literature that asphyxia increases the rate of output of epinephrin apparently accounts for the uncritical way in which he finds support in his own observations for the view that increased adrenalin output is a considerable factor in asphyxial hyperglycemia. He seems to forget that it would not be enough to prove that asphyxia causes an increase in the epinephrin output, it must be shown that the increase is sufficient to bring the epinephrin content of the blood to the level necessary for adrenalin hyperglycemia and to maintain it there.

In our own work (7), (8), (9), we have not found evidence that there is any detectable increase in the output per unit of time, although, of course, when the blood flow through the adrenals is slowed the concentration of epinephrin in it is increased. The reason why our result differs from that of Kellaway is that we used quantitative methods which really enabled us to measure the output of epinephrin whereas he did not make any measurements at all, but assumed from the effect of asphyxia in causing the paradoxical pupillary reaction that there must have been an increase in the output. This is not a specific qualitative reaction for adrenalin, let alone a reaction by which the rate of output can be quantitatively estimated.² The fundamental difference

² The pupil reaction can be utilized for estimating quantitatively the epinephrin in adrenal vein blood, and it has been so employed by us. But to do this variations in the other factors which may affect the pupil (anesthesia, asphyxia, etc.) must be eliminated, the only change made being the addition to the general blood of the epinephrin containing-adrenal blood collected in a cava pocket for a definite time or the adrenalin artificially injected to assay it.

between Doctor Kellaway's work and our own embraces much more than the single question of the effect of asphyxia on the epinephrin output. All our work on the influence of various conditions and various substances upon the epinephrin output has aimed at a quantitative determination of epinephrin in the adrenal vein blood. Being able to tell how much epinephrin the adrenals were giving off per minute before the factor under investigation was allowed to act, and how much they were giving off while it was acting, we were naturally in a different position for determining whether any change had occurred from that occupied by an observer who could not have any idea how much epinephrin was being given off at any time throughout his experiment.

He has shown that the anoxemia is the important factor in producing the paradoxical dilatation of the pupil in asphyxia. This is a new and interesting point. By graduating the severity of the asphyxia, as he terms it, i.e., by causing the animal to breathe mixtures with definite percentages of oxygen less than that of the atmosphere, he has satisfied himself that after interference with the epinephrin output it is less easy to provoke the paradoxical dilatation than in normal animals, although in non-anesthetized animals he still gets a fair reaction. He studied the difference produced in this reaction in cats by section of the splanchnics in survival experiments and by removal of both adrenals. We have no observations on cats after bilateral splanchnotomy. Our animals were prepared by excision of one adrenal and section of the nerves of the other according to Elliott's method, and we were unable to convince ourselves, as we have stated in a previous paper (10) "that there is any striking difference" in the paradoxical reaction induced by asphyxia in these animals as compared with normal cats, although "we should rather expect a difference if the normal epinephrin output," as we believe, "is already exerting an action" on the sensitised iris. Kellaway also obtained a relatively small difference between the normal and operated cats with the severer grades of asphyxia, and it is possible that by using his "graduated" method instead of the cruder methods previously employed a similar difference would be made out in cats prepared in the way mentioned as he found in cats after section of the splanchnics. But this, so far as we can see, would indicate merely that the epinephrin was a factor in the asphyxial paradoxical reaction and would not prove that the output was *augmented* by asphyxia.

We have obtained evidence (10) that epinephrin passing into the blood at the ordinary rate under the conditions of our experiments exerts an action on the pupil (after removal of the superior cervical ganglion). A dilatation produced by epinephrin disappears more quickly if the adrenal blood is prevented from entering the circulation. In a previous paper (9) we say that this being so "there is every reason to expect that asphyxia, which even according to Kellaway causes some paradoxical dilatation in the absence of the adrenals, will increase the reactivity of the pupil to this ordinary output." Kellaway professes to find that the phrase "every reason to expect" "does not appear to have any definite

significance." We should have thought it self-evident that since asphyxia, as such, that is to say in the absence of the adrenal epinephrin, can cause dilatation of the sensitised pupil and since epinephrin can also cause dilatation there would be every reason to expect that a given output of epinephrin would cause a greater effect when favored or reinforced by the action of asphyxia than in the absence of asphyxia, and that accordingly the fact that a paradoxical reaction was more easily elicited or elicited in greater strength with intact adrenals than in their absence would not of itself show that asphyxia augments the rate of output of epinephrin.

We have observed in non-anesthetised cats (some days after transection of the cord in the cervical region) that the giving of ether increased the reactivity of the pupil, sensitised by previous removal of the superior cervical ganglion, to adrenal vein blood collected in a cava pocket, so that with a given time of collection a reaction was obtained where none had been obtained before, or a good reaction was elicited where only a small one had been got before the ether. At first sight this looks like a proof that ether augments the epinephrin output even after cervical cord section. And a good many statements with no definite basis exist in the literature to the effect that anesthetics cause an accelerated output. All that was necessary to dispose of this interpretation of the experiment mentioned, was to inject a definite dose of adrenalin before and after administration of ether. The pupillary response to one and the same dose was increased by the ether. The explanation we think is obviously that ether, which itself causes a dilatation of the sensitised pupil, favors the dilating action of the adrenalin, just as asphyxia does. Depression of the pupillo-constrictor activity, however produced, would bring about such an effect, and Langley (11) points out that paralysis of the ciliary ganglion by nicotine, for example, may complicate observations made by the aid of the pupillary paradoxical reaction. Kellaway's experiments on the excised iris throw no light upon the question.

His observation that when the aorta is clipped asphyxia causes dilatation of both pupils, whereas on releasing the aorta there is a preferential dilatation of the sensitised pupil simply shows that enough epinephrin secreted at the ordinary rate has been collected in the adrenal vessels and the cava to give a good or a maximal pupillary paradox when it is allowed to move on after release of the aorta. The experiment is only a repetition in a crude form of our own observations on the measurement of the epinephrin output by the pupil reaction, and the paradoxical reaction is also obtained without asphyxia.

It is a complete mistake to impute our negative result in asphyxia to the condition of the animals in consequence of the operation practised by us to obtain the adrenal vein blood. *The paradoxical pupillary reaction is excellently obtained at the time when we are inducing asphyxia after having prepared the cava pocket for collection of the adrenal blood and thereafter in the course of the experiment.* According to Kellaway this proves that the epinephrin output is augmented. We collect the

adrenal blood at this time. We estimate the concentration of epinephrin in the blood and, knowing the rate at which the blood was collected, we calculate the output of epinephrin per minute and we find that the output has not been changed by the asphyxia. The "clear and logical conclusion" is not that the phenomenon of the acceleration of adrenalin secretion supposed to be evidenced by the pupillary reaction fails under the conditions of our experiments, but that no sensible acceleration is produced by such grades of asphyxia as we have employed.

This ought to be sufficient to dispose of the suggestion that the operative procedure adopted to obtain the adrenal blood vitiates our results. But a few words on the development of the technique may prevent misunderstanding. Permanent ligation of the abdominal aorta just above the bifurcation, of the renal arteries and veins and of the inferior cava was not practised until it was shown that it did not cause any demonstrable effect on the epinephrin output. In one of our earlier papers (7) it is stated under "technique" that "where the eye reactions are used alone the cava pocket need not be permanent. For certain purposes the temporary closing off of the pocket for a minute or two at a time is all that is necessary, and in the interval the circulation proceeds practically in the normal way. A clamp is applied just above the iliac veins. The renal veins are then clamped and the segment of cava emptied of blood by gently stripping it upwards. Finally a clamp is put on the cava above the adrenal veins. Only a few seconds are occupied in the adjustment of these clamps. Small veins entering the cava segment have been previously tied." It was then tested whether permanent ligation of these vessels made any difference in the results. No difference having been found we thereafter tied these vessels as a matter of routine, and in our papers where it is simply stated that "the cava pocket was formed" it is implied that the vessels tied were only the abdominal aorta, cava, renal arteries and veins and the small veins. For blood pressure assays it was sometimes found advantageous to tie the superior mesenteric artery and the coeliac axis to eliminate irregularities in the curve. Before this was done numerous observations were made which failed to reveal that the epinephrin output was at all affected by this procedure. We have continued to tie these vessels or sometimes only the superior mesenteric, in some of our experiments, because a higher blood pressure and better blood flow through the adrenals and their nervous mechanism are thus insured. Their ligation is not in any way inherent in our method and in all our investigations there are plenty of experiments in which they were not tied, for example, the last experiment on asphyxia published by us (9). In our large series of experiments on strychnine, nicotine and other drugs the superior mesenteric artery and coeliac axis were not ligated. We have also measured the epinephrin output in blood collected from one adrenal of a dog through a lumbar incision without opening the peritoneum and found no essential difference in the results from those of our other experiments.

SUMMARY

1. Our previous conclusion that the adrenal epinephrin output is not essentially concerned in the hyperglycemia induced by ether narcosis and asphyxia is confirmed. In rabbits which have survived the removal of both adrenals and have recovered from the operation and whose livers are well filled with glycogen, hyperglycemia is caused by these procedures just as in normal rabbits. We reaffirm our position that for these forms of experimental hyperglycemia and for the hyperglycemia caused by piqûre the so-called adrenalin hypothesis should be abandoned.

2. We do not find that any real evidence has been adduced by Kellaway that the epinephrin from the adrenals has a demonstrable share in the production of asphyxial hyperglycemia. His experiments really confirm our conclusion that interference with the epinephrin output does not modify essentially the hyperglycemia caused by asphyxia.

3. Kellaway has produced no evidence that asphyxia (or anoxemia) causes a demonstrable increase in the rate of epinephrin output from the adrenals. Despite his statement that "the results of the different experiments fully justify the conclusion that the paradoxical pupil reaction is a good index of the epinephrin output," we do not believe that by looking at the eye of an intact cat he can tell the amount of epinephrin coming off from the adrenals per unit of time before, during or after asphyxia, whereas we can obtain these amounts by collecting adrenal vein blood and assaying its content of epinephrin. The real reason for the difference in our conclusions as to the influence of asphyxia upon the rate of epinephrin output is that we have attacked a quantitative problem by direct quantitative methods instead of trusting to ambiguous reactions which are not even specific qualitative reactions for epinephrin. Our data, therefore, have a very different value for the determination of changes in the rate of epinephrin output.

4. Kellaway is completely mistaken in imputing our results on the epinephrin output in asphyxia to the condition of the animals in consequence of the operation practised by us to collect the adrenal vein blood. The paradoxical pupillary reaction is excellently obtained at the time when we are inducing asphyxia after having prepared the cava pocket for collection of the adrenal blood and thereafter in the course of the experiment. According to Kellaway this proves that the epinephrin output is augmented at the very time when we are collecting the blood. We ought therefore to be able to detect the augmentation

by our method. Since we do not detect an increased output by assaying the very blood in which the epinephrin is carried we are compelled to conclude that Kellaway's interpretation of the pupillary reaction as demonstrating an increased output of epinephrin in asphyxia is erroneous.

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THE ACTION OF DRUGS UPON THE OUTPUT OF EPINEPHRIN FROM THE ADRENALS I. STRYCHNINE

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We have already more than once emphasized the fact that the spontaneous liberation of epinephrin is not easily influenced by experimental conditions. The relatively great stability of the rate of output made it likely that if drugs are capable of affecting the output in any marked degree there would be no special difficulty in detecting and estimating the amount of the change. We began with strychnine, because it seemed probable that a drug which caused so marked an effect on the motor mechanisms of the cord would also exert an action upon the mechanism which is associated with the liberation of epinephrin from the adrenals. We had previously found that the injection into the blood stream of small quantities of concentrated solutions of salts (sodium carbonate), which, as is known, causes a general excitation of the cord, with convulsions, was followed by a transient but marked increase in the epinephrin output.

DISCUSSION OF ESSENTIALS IN THE TECHNIQUE OF MEASURING THE EPINEPHRIN OUTPUT

Many of the statements in the literature upon the effect of drugs and other substances on the epinephrin output are based upon experiments by inadequate methods. It is not merely that some writers habitually interpret changes in the epinephrin store of the adrenals in terms of changes in the rate of liberation of epinephrin, but even when it has been recognized that it is the changes in the blood coming from the gland

which it is essential to investigate it has often been strangely overlooked that when the concentration of epinephrin in the drawn blood has been estimated it is still necessary to know the rate of the blood flow through the adrenals in order to arrive at the rate of output. How little this matter, elementary as it may seem, has been understood, appears from the fact that one of the methods most frequently employed to study changes in the epinephrin output (collection of blood by a catheter from the inferior cava above the level of the adrenal veins (1), (2), and subsequent application of the blood to intestine strips or segments) does not permit the estimation of the flow through the adrenals or even in the cava. We say nothing of the fact that the adrenal blood is diluted greatly and probably in a different degree in successive observations, with the ordinary venous blood. Even those investigators who have recognized that the concentration of epinephrin in adrenal vein blood being at best small it is desirable to work with pure adrenal blood, have not always realized that the rate of flow is an indispensable factor for the determination by such methods of the rate of output. Gley and Quinquaud, for example, in a recent paper (3) remark that in asphyxia the output of epinephrin is increased, since they have found that two or three times as much adrenal blood collected from a dog, without asphyxia, must be injected into another dog to cause a given rise of blood pressure as of adrenal blood collected during asphyxia lasting two to four minutes. That they have taken no account of the rate of the blood flow is obvious since they bring forward as confirmatory evidence of an increased output the fact that a given amount of blood collected from the inferior cava above the adrenals without asphyxia causes a smaller rise of pressure in another dog than an equal amount collected during asphyxia. Here they could not possibly have measured the rate of flow.

They conclude, nevertheless, that the quantities of epinephrin liberated are too small to cause any demonstrable reaction in the organism, since they cannot detect epinephrin in the blood of the right heart even when the output is increased by stimulation of the peripheral end of the splanchnic nerve. We believe that these writers are mistaken in both conclusions: asphyxia, at any rate for such periods and with such methods as we have employed, does not produce a demonstrable increase in the output of epinephrin, but if it did definite reactions could easily be caused by it. It has been shown, as a matter of fact, by Joseph and Meltzer (4) that stimulation of the splanchnic causes dilatation of the pupil on the side on which the superior cervical ganglion has been previously

excised. Their interpretation of this dilatation as due to increased epinephrin liberation from the adrenals has been abundantly confirmed by Elliott (5), Stewart, Rogoff and Gibson (6), and others. We have even obtained evidence that the amount of epinephrin spontaneously liberated is not without effect upon the denervated iris and other structures (7). The only reason for the failure of Gley and Quinquaud to demonstrate epinephrin in the blood of the right heart is that the reaction used by them (the rise of blood pressure produced in one dog by injection of blood from another) although in other respects a good reaction, is not delicate enough.

Their supposed proof of an increased output of epinephrin in asphyxia is nothing more than a proof of an increased concentration of epinephrin in the adrenal vein (or cava) blood. Such an increased concentration would necessarily occur, if the rate of output per minute remained unaltered, provided that the average blood flow through the adrenals (or in the cava) was diminished by prolonged asphyxia. This is precisely what we have found to occur, as shown in the following experiment.

Condensed protocol; dog 307; female; weight, 4.6 kgm.

Anesthetized with morphine and ether. Obtained a specimen of indifferent blood from external jugular vein. Made cava pocket. Then collected adrenal blood.

- 11.44 a.m. First specimen, 4.25 grams in 30 seconds (8.5 grams per minute).
- 11.44½ a.m. Second specimen, 11.05 grams in 90 seconds (7.4 grams per minute). Blood pressure during collection of adrenal specimen was 90 mm. Hg.
- 11.50 a.m. Total asphyxia started. Then collected adrenal blood.
- 11.50½ a.m. Third specimen, 2.9 grams in 60 seconds (2.9 grams per minute).
- 11.51½ a.m. Fourth specimen, 5.75 grams in 90 seconds (3.8 grams per minute). Blood pressure towards end of collection of fourth specimen 72 mm. Hg.
- 11.53 a.m. Stopped asphyxia for a short period (20 to 30 seconds).
- 11.53 a.m. Fifth specimen, 9.2 grams in 120 seconds (4.6 grams per minute).
- 11.53½ a.m. Started total asphyxia.

- 11.55 a.m. Sixth specimen, 7.6 grams in 120 seconds (3.8 grams per minute). Blood pressure during collection of sixth specimen 62 mm. Hg.
- 11.57 a.m. Started artificial respiration.
- 12.05 p.m. Seventh specimen, 9.45 grams in 120 seconds (4.7 grams per minute).
- 12.07 p.m. Eighth specimen, 9.75 grams in 120 seconds (4.9 grams per minute). Blood pressure during collection of eighth specimen 60 mm. Hg.
- 12.08 p.m. Started total asphyxia.
- 12.10 p.m. Ninth specimen, 3.5 grams in 120 seconds (1.8 grams per minute). Blood pressure during collection of ninth specimen 56 mm. Hg.
- 12.12 p.m. Started artificial respiration and obtained abdominal aorta (indifferent) blood.
- Combined weight of adrenals 0.831 gram.

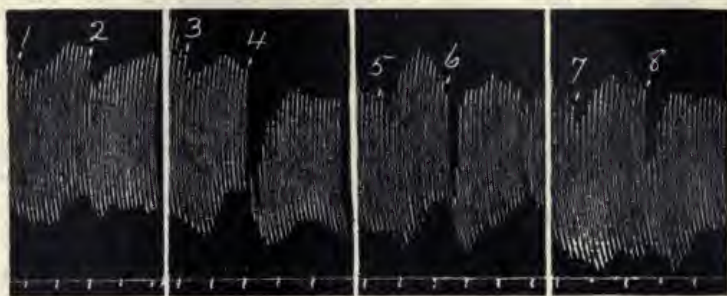


FIG. 1. INTESTINE TRACINGS. BLOOD FROM DOG 307

At 1, 3, 5, and 7 Ringer was replaced by indifferent (jugular) blood, and this at 2, by the second adrenal specimen (collected before asphyxia); at 4 by the sixth specimen (collected during and after prolonged asphyxia); at 6 by the eighth specimen (collected eight minutes after end of asphyxial period); at 8 by the second specimen. All the bloods were diluted with three volumes Ringer. As in all figures showing intestine or uterus tracings, time is marked in half minutes. (Reduced to one-half.)

The second adrenal specimen (taken before asphyxia) had a smaller concentration of epinephrin than the sixth (taken during and after prolonged asphyxia). The eighth specimen (collected after artificial respiration had been going on for some time) was intermediate in strength. This is well illustrated in figure 1. At this stage the intestine segment

was not very sensitive to epinephrin, so that little more than a just detectable reaction was given by the second specimen (observation 2) the first time it was applied to the segment. A little later the second specimen gave a somewhat greater reaction (observation 8). A reader unfamiliar with these methods of assay might have supposed from a simple comparison of observations 2 and 4 that the output of epinephrin must have been increased when the asphyxia specimen was being collected. Indeed, some of the statements in the literature to this effect are based on no better evidence. The truth is, of course, that here the sensitiveness of the segment was such as just to permit the detection of the epinephrin concentration in the second specimen. Had the segment been a little less sensitive, the second specimen would have given no

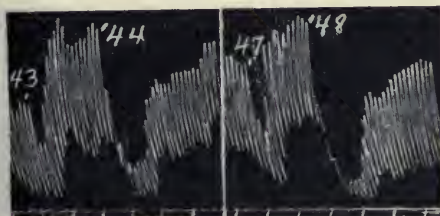


FIG. 2. INTESTINE TRACINGS. BLOODS FROM DOG 307

At 43 and 47 Ringer was replaced by indifferent (jugular) blood, and this at 44 and 48 by the second and sixth adrenal specimens (collected before and during prolonged asphyxia) respectively. The bloods were diluted with three volumes Ringer. (Reduced to one-half.)

reaction, while the sixth would have given a good reaction and the conclusion might have been drawn by a novice that an outburst of epinephrin had occurred during the period of asphyxia. The assay showed, as a matter of fact, that the output was not at all augmented. Later on, the sensitiveness of the segment being increased, the reaction caused by the second specimen was much greater, but the preponderance of the sixth was still maintained (fig. 2, observations 44 and 48). The eighth specimen also occupied the same relative position, being weaker than the sixth (fig. 3, observations 54 and 60). The ninth specimen, collected during a further period of asphyxia and with a much smaller blood flow than any of the others, had, of course, the greatest concentration of all (fig. 3, observation 56).

Observations on a uterus segment demonstrated that the various adrenal specimens caused increases in the tone of the preparation cor-

responding to the inhibitory effects produced on the intestine segment, indicating that the inhibition was due to epinephrin and not to other substances said sometimes to be present in the general blood stream in asphyxia which cause a diminution of tone in both rabbit intestine and uterus.

The epinephrin assay showed that the second adrenal specimen (collected before asphyxia) was much weaker than 1:1,500,000 adrenalin, weaker than 1:3,000,000, a little weaker than 1:4,500,000, much stronger than 1:7,500,000, decidedly stronger than 1:6,000,000, probably somewhat stronger than 1:5,200,000. It was finally taken at 1:4,800,000, corresponding to an output of 0.0015 mgm. per minute for the dog, or 0.00033 mgm. per kilogram per minute.

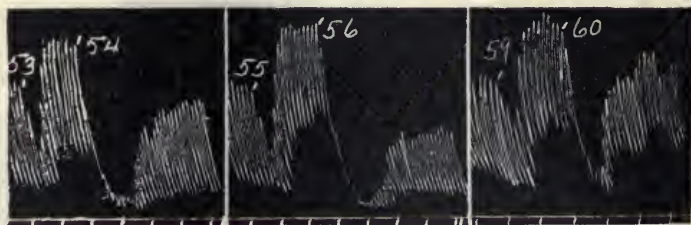


FIG. 3. INTESTINE TRACINGS. BLOOD FROM DOG 307

At 53, 55, and 59 Ringer was replaced by indifferent (jugular) blood, and this at 54 by the sixth adrenal specimen (asphyxia); at 56 by the ninth (asphyxia); at 60 by the eighth specimen (collected eight minutes after end of an asphyxial period). The bloods were all diluted with three volumes Ringer. (Reduced to one-half.)

The sixth specimen (collected during and after prolonged asphyxia) was decidedly weaker than 1:1,500,000, and very nearly equal to 1:2,200,000, probably slightly weaker. Taking it at 1:2,300,000 we get 0.0016 mgm. per minute for the dog or 0.00035 mgm. per kilogram per minute, the same as for the second specimen.

The eighth specimen (collected after a period of artificial respiration) was assayed at 1:3,000,000, corresponding to an output of 0.0016 mgm. per minute for the dog, or 0.00035 mgm. per kilogram per minute.

The ninth specimen (collected during asphyxia) was found to be weaker than 1:1,100,000, a little stronger than 1:1,500,000. It was taken as 1:1,300,000, corresponding to an output of 0.0014 mgm. per minute for the dog or 0.0003 mgm. per kilogram per minute. Obviously, notwithstanding the great increase in the concentration, the out-

put remained unchanged during asphyxia. The proportion of serum was 62 per cent, determined by the electrical method (8). The concentration of epinephrin in the serum of this specimen was accordingly 1:800,000, considerably less than the "possible normal maximum."¹

We have previously shown (9) that asphyxia does not increase the output, although when pushed to the point at which it diminishes the adrenal flow, it increases the concentration of epinephrin in the adrenal blood. A slowing of the blood flow produced in any other way, as by hemorrhage, inhibition of the heart by vagus stimulation, impairment of the heart or of the vasoconstrictor mechanism is associated with a similar increase in the concentration of epinephrin in the adrenal vein blood. From an increased concentration nothing whatever can be deduced as to an increase in the rate of output of epinephrin, unless it is known that the blood flow has not been correspondingly decreased. The matter is precisely on the same footing as the measurement of the rate of production of carbon dioxide by a tissue or organ, by determining the carbon dioxide content of the incoming and outgoing blood. Who would conclude from the fact that the carbon dioxide content of venous blood coming from the hind legs of an animal was increased relatively to that in the arterial blood, when the arterial pressure was lowered by stimulation of the vagus or by such a poison as nicotine, that the metabolism of the hind legs had been increased by the stimulation of certain fibres in the vagus or by the poison? Would it not be quite clear that the carbon dioxide production per minute had not been determined by estimating merely the carbon dioxide content of the blood and that in addition a measurement of the rate of blood flow was necessary? In this case it would be found that the increase in the excess of carbon dioxide content of venous as compared with arterial blood was just balanced by the decreased blood flow and that there was no increase in the output. To conclude from an observed increase in the epinephrin concentration in the blood coming from the adrenals that the output per minute has been increased, without information as to changes in the rate of blood flow is quite as unjustifiable as to come to a similar conclusion in regard to carbon dioxide. Whether epinephrin is a

¹ By the "possible normal maximum concentration" is meant the maximum concentration of epinephrin (assayed by rabbit's intestine and uterus segments), actually observed in the blood or serum in adrenal blood specimens collected with the slowest blood flows in animals anaesthetised with morphine, ether, or urethane. When this maximum concentration has been reached it is obvious that the output of epinephrin cannot be increased unless the blood flow is increased.

product of the metabolism of the adrenal medulla which is simply being got rid of, or a secretion with a function in the organism has, of course, nothing to do with the question.

It is the same with the experiments from which Gley and Quinquaud (*Arch. internat. de Physiol.*, 1914, xiv, 152) deduce the conclusion that relatively large doses of extracts of thyroid and other glands (pancreas, liver, etc.) increase the output of epinephrin when injected intravenously. These extracts are known to lower the blood pressure, and a figure is even given by the French authors showing this in the case of an injection of liver extract. The adrenal blood flow may, therefore, be assumed to have been diminished during collection of the sample of blood which is supposed to demonstrate the increased epinephrin output, and the concentration of epinephrin in it must have been increased. Yet because a given quantity of this blood produces a greater rise of pressure in another dog than an equal quantity of the "normal" adrenal blood it is concluded that the rate of output of epinephrin has been increased by the organ extract. Quinquaud (*Trav. du lab. de biol. gén. de Collège de France*, 1915)—has been misled by want of attention to the same point in his conclusion that piqûre increases the epinephrin output.

It ought to be emphasized that the method used by these observers is adequate for determining the concentration of epinephrin in the adrenal vein blood. The results are vitiated merely by the failure to take account of the rate of adrenal blood flow. Quantitative conclusions have, however, been drawn not infrequently from methods which are unsuited to yield quantitative information.

Quite recently Kellaway (*Journ. of Physiol.*, 1919, lii, 63) has stated that "the paradoxical reaction of the pupil to asphyxia is wholly due to anoxaemia and mainly to . . . accelerated secretion from the adrenal glands." Even granting that anoxaemia produces constantly a much smaller dilatation of the pupil (sensitized by previous removal of the superior cervical ganglion) when the adrenals have been excised or the splanchnic nerves cut than with intact adrenals, all that ought to be deduced from this is that the epinephrin coming from the adrenals is concerned in the dilatation of the "sensitized" pupil. The epinephrin discharged at the ordinary rate is known to exert an action on the sensitized pupil and there is every reason to expect that asphyxia, which even according to Kellaway causes some dilatation in the absence of the adrenals, will increase the reactivity of the pupil to this ordinary output. Changes in the circulation

associated with asphyxia may also increase the blood flow through the sensitized iris and therefore the amount of epinephrin passing through it per unit of time. Such circulatory changes may also alter the concentration of epinephrin in the inferior cava without any alteration whatever having occurred in the rate of output. All these sources of error are eliminated by methods which permit the collection of adrenal vein blood and its assay on test objects which have not themselves been subjected to such conditions as asphyxia.

As a matter of fact, the response of the sensitized pupil to asphyxia varies quantitatively to a considerable extent in different cats after interference with the epinephrin discharge and also varies according to the degree of the asphyxia. But this is also true of normal cats, and we have never been able to convince ourselves that there is any striking difference. It may be that a very large series of observations would bring out some difference and this we should rather expect if the normal epinephrin output is already exerting an action. Possibly also the more gradual diminution of the oxygen in the blood when an animal is caused to breathe a gaseous mixture with a constant although diminished oxygen content, may cause a somewhat different effect from that of total asphyxia suddenly produced. We are certain, however, that one or two observations possess no value in a question of this kind. As already stated, such observations are in any case quite unsuitable for determining whether changes in the rate of epinephrin output are occurring or not. When we began our work on asphyxia it was with the expectation that so general an excitant of nervous centers would be found to excite the epinephrin secretory mechanism. But hitherto we have never been able to demonstrate such an action. Whether in an animal dying in asphyxial convulsions the central mechanism governing the liberation of epinephrin might be stimulated along with other centers with a resultant increase in the rate of epinephrin output, if the adrenal medulla was still capable of responding to the stimulation, is unknown.

An obvious fallacy underlies the conclusion of Roger (J. de Physiol. et Path. gén., 1917, xvii, 187) that because the cardiac inhibition, produced by stimulating the peripheral end of the cut vagus is less durable in normal than in adrenalectomized rabbits, in the former the inhibition must be cut short by the action of an *augmented* epinephrin discharge upon the heart. The observations, assuming their accuracy, can only show at most that the epinephrin coming from the adrenals exerts an action upon the heart which terminates the in-

hibition sooner than when epinephrin is absent. They may constitute, indeed, a proof that the normal output of epinephrin exerts a demonstrable effect upon the heart, of which we possess other proofs. But they cannot possibly demonstrate an *increased* output.

Technique. Blood was collected in the usual way from a cannula inserted into a pocket of the inferior cava. In making the pocket all veins entering the cava below the level of the liver, except the adrenal veins were tied. We always perform the operation in such a way that no leakage of any blood takes place into the pocket, except from the adrenals. In our experiments we are certain that only pure adrenal blood was collected, both in dogs and cats. The abdominal aorta was ligated near the bifurcation. The renal with the spermatic or ovarian arteries were also tied. In collecting the adrenal blood samples, a preliminary specimen was taken first, in order to wash out any epinephrin possibly liberated in the manipulation. The total dead space in cannula and vein in a cat is about 0.5 to 1 cc. In the rather small dogs employed in these experiments the dead space is about 1 to 2 cc. A fresh cannula (boiled and oiled) is always inserted after the collection of each pair of specimens. The cannula and vein are empty at the moment when the pocket is clamped off above the level of the right adrenal vein, and the time of collection of the first specimen is reckoned from the moment when blood begins to drop into the dish. The true time at which the blood began to leave the adrenals is obtained approximately by correcting for the blood required to fill the dead space at the given rate of flow. This is only necessary when it is required to fix more exactly the time interval between the introduction of a new factor in the experiment, for example, the injection of a drug, and the beginning of the resultant change in the epinephrin secretion.

The blood specimens were defibrinated and kept on ice till the assay on rabbit intestine (and uterus) segments was made. The assay was begun in general within an hour of obtaining the last specimen. In a very few instances it was necessary to leave the completion of the assay of one or two of the samples till the following morning. When this was inevitable a provisional assay was made on the day on which the blood was drawn. Dale and Laidlaw (10) have asserted that defibrinated blood is not suitable for assaying epinephrin on intestine strips or segments, because of the presence of a substance developed in clotting which exerts a tone-increasing effect on the unstriped muscle. They recommend the injection of hirudin into the blood to prevent clotting. We have no objection to the use of hirudin to prevent clots in the can-

nula, although we believe it is better to insure against this by inserting fresh cannulae and avoid injection of hirudin at least until it is proved that it has no effect on the epinephrin output. Also hirudin itself is capable of causing some effect on the test segments. But we are certain that it is an error to suppose that it is a disadvantage that the blood should cause an increase of tone in the segment. We believe, on the contrary, that it is a distinct advantage, as the tone-decreasing power of the epinephrin-containing blood when it displaces the indifferent blood has a better opportunity of manifesting itself when the tone has been previously increased. In our work we prefer that the indifferent blood should cause a good increase of tone in the segment, and we have often observed that a segment which at first responded to the blood by only a small increase of tone or none at all, and was not very sensitive to epinephrin, became decidedly more sensitive later on when its tone was increased to a greater extent by the indifferent blood.

In any case, as was shown by Stewart (11) and by Stewart and Zucker (12) the difference in the action of plasma and serum is much more conspicuous, more constant and more easily elicited in the case of vascular objects, like artery rings or perfused frogs' legs than in the case of rabbit intestine or uterus segments. With undiluted or only moderately diluted heterologous blood, little if any difference was found in the tone-increasing effect of the unclotted and defibrinated blood, or of the plasma and serum. The main object of that work was to determine a question of considerable importance in the technique of testing for epinephrin in blood, namely whether any advantage was to be gained by using unclotted blood or plasma for application to rabbit intestine and uterus segments, rather than the much more easily obtained defibrinated blood or serum. For this reason, only such kinds of blood (almost always heterologous) and such dilutions (never very great, especially in the case of the intestine) were employed as were most commonly used in the epinephrin determinations. It was clearly pointed out that our results "need not imply that the pressor substance, if it is a single definite substance developed in the shed blood, exerts no action on the smooth muscle of the intestine and uterus preparations, but merely that its action on these objects is masked by the general action of the serum and plasma, so that, in the presence of the other constituents common to serum and plasma, its effect is inconspicuous or not to be detected at all, while on the blood vessel preparations, especially the artery rings, the effect of the pressor substance is the dominant one, and the general action of the serum and plasma is feeble or undetectable."

It must be remembered that an intestine or uterus segment is a very complex structure compared with an artery ring. It would be nothing short of miraculous if a segment which shows itself so sensitive to slight changes in the composition of an artificial fluid in which it is beating, and to changes in the oxygen supply should be quite unaffected when Ringer's solution is replaced by such a liquid as hirudin plasma or unclotted hirudin blood. As a matter of fact, we found that such liquids as hydrocele fluid and ascitic fluid, which had never clotted and were incapable of spontaneous coagulation, invariably caused the same qualitative effects as serum on rabbit intestine or uterus segments, although, quite inert as regards artery rings. Serum which had lost its power of constricting artery rings (13) after digestion with blood vessels (Tatum (14)) and after being subjected to other processes still caused a marked increase in the tone of intestine and uterus segments. There is some reason to think that the specific action of the serum on smooth muscle may be more evident as compared with its general action on the segments when the serum is considerably diluted. We, ourselves, saw and have figured instances of this, as already stated. In most of our experiments the serum and plasma, or the defibrinated and unclotted blood were either undiluted or only moderately diluted and here the general action common to both preponderated. Where a small amount of blood is run into a relatively large vessel of Ringer's or Tyrode's solution, as was done by Dittler (15), the difference in the action of serum and plasma may, therefore, become more conspicuous than in our observations.

Another fact must be carefully kept in mind. We have several times pointed out that in general the first applications of blood or serum to a segment, especially to an intestine segment, will produce an increase of tone which is less, often much less, than it will be in subsequent applications. For this reason, in our epinephrin assays we never begin work with the adrenal bloods until the segment has been once or twice subjected to the action of the indifferent blood. If now, on comparing the action of serum and plasma, the plasma be added to a fresh segment, the effect will usually be decidedly less than that of serum when this is added later, or there may appear to be little, if any, effect. It is always necessary in such comparisons to make numerous observations and to vary the order in which the liquids are applied to the segment. This circumstance must also be taken into account in such experiments as those of Dittler, in which arterial blood is run directly into the vessel where a fresh segment is beating in the artificial saline medium. It may

be expected that the period which elapses before the segment reacts will be longer for this reason if for no other, than when in a subsequent observation, defibrinated blood is similarly applied to the segment. It is not suggested that the whole of the difference between unclotted and clotted blood observed by Dittler can be explained in this way, but this factor must be taken into account.

It must further be considered that in Dittler's experiments the unclotted blood was always applied to intestine segments from the same animal, while this was not always the case with the serum or defibrinated blood. In our experiments indications were observed that differences between plasma and serum (or unclotted and defibrinated blood) were more likely to exist and to be of greater magnitude in the case of homologous than in the case of heterologous blood. As the latter has been invariably employed in our epinephrin assays, this is an additional reason for not attempting at the cost of complicating the experiments considerably to gain a theoretical advantage by using unclotted instead of defibrinated blood. A more important contraindication is that even if it were admitted that when great precautions are taken unclotted blood can be obtained from other animals as well as from rabbits, which in considerable dilution will be without action on rabbit segments, there is no guarantee that this blood will not clot and develop its pressor effect in a manner quite beyond control in contact with the segment.

O'Connor (16) indeed, who maintains that the tone-increasing action of blood on the intestine is entirely developed in clotting says that this is very difficult to demonstrate for the precise reason that in the presence of the thromboplastic substances in the intestine segment clotting may occur. If this were true, it is obvious that with the use of plasma in such long series of observations as are practised by us in assaying epinephrin in adrenal blood, the reaction of the segment to the indifferent blood and, therefore, the extent of the inhibition produced by the epinephrin-containing blood might be expected to vary in a manner which could not be controlled in successive observations. On the other hand, the increase of tone produced by a specimen of defibrinated blood is sufficiently constant to allow numerous comparative observations to be made on the same segment.

As in our experience, rabbit intestine (and uterus) segments constitute the best objects at present known for the assay of epinephrin in blood, we have entered at some length into the question, probably the most important in the whole technique, whether it is necessary to use unclotted blood or plasma. We have no hesitation in concluding that far

from being necessary, it is not advantageous to attempt to do so. Defibrinated blood (or serum) is better. Writers who have suggested that the results obtained on intestine segments or strips were vitiated by the fact that defibrinated blood or serum was employed have not themselves had any experience of the method, and have been misled by the fact that for the vascular test objects the conditions are quite different. Thus, for tests with artery rings unclotted blood (or plasma) is indispensable, while for the frog perfusion preparation it is probably considerably superior to defibrinated blood (or serum).

We regret the necessity of discussing at such length these questions of technique. But most of the confusion and uncertainty in the literature of the subject is due to the misinterpretation of results obtained by methods which have been applied without adequate examination and criticism. A discussion of methods seems therefore an almost indispensable preamble to a series of papers purporting to examine the action of drugs upon the output of epinephrin. We return now to our experiments.

While in the case of strychnine and the other drugs examined we relied mainly on epinephrin assays with rabbit segments, corroborative evidence was sought: (a) By comparing the rise of blood pressure produced by adrenal blood collected for a definite time in a cava pocket and then released, before and after administration of the drug. (b) By studying the effects of the drug on the eye after excision of the superior cervical ganglion, in normal cats and in cats whose epinephrin output had been interfered with by removal of one adrenal and section of the nerves of the other.

The preference must always be given, we believe, to methods in which blood is collected directly from the adrenals and the epinephrin content of the drawn blood then assayed on suitable test objects. Next comes the method in which the adrenal blood is collected for a given time in a cava pocket and then released into the circulation of the animal, where it produces some definite effect as on the blood pressure or the structures of the denervated eye, the amount of which effect can be estimated. An obvious limitation of this method of auto-assay is that procedures whose effect on the epinephrin output is being studied may themselves alter the sensitiveness of the test objects.

The mere comparison in the intact animal of the effect of drugs on such objects as the denervated eye with the adrenals discharging epinephrin and when the discharge has been suppressed, can only be used in general to corroborate results obtained by more direct methods.

Observations of this kind must be interpreted with great care if deductions are to be drawn as to any effect produced on the epinephrin output. Many errors have arisen from the uncritical use of such observations, which at best can seldom yield reliable quantitative results.

A few experiments were made on the influence of some of the drugs on the epinephrin store of the adrenals.

EXPERIMENTS ON DOGS

We began purposely with large doses in order to produce a marked effect. Later on the effect of smaller doses was studied. No attempt is made in this paper to fix the minimum effective dose, which must in any case vary with the anaesthetic and other circumstances, but doses lying well within the therapeutic range were found to produce decided increases in the epinephrin output.

Some typical experiments on dogs, with condensed protocols and specimens of the tracings used in the epinephrin assay, will now be given. As for each animal, on the average, the epinephrin assay involved at least 30 to 40 separate applications to the segments, of adrenal blood or indifferent blood made up with adrenalin, it is obviously impossible to reproduce a sufficient number of tracings to give a complete picture of the assay in even one animal. We have, therefore, judged it best to illustrate pretty fully the assay in one experiment and for the others to give only enough tracings to bring out special points. In the experiment chosen the strychnine effect was neither the largest nor the smallest observed.

Condensed protocol; dog 257; female; weight, 6.0 kgm.

Anesthetized with morphine and ether. Obtained a specimen of indifferent blood from external jugular vein. Made cava pocket. Blood pressure 118 mm. Hg. Collected adrenal blood.

11.20 a.m. First specimen, 4.65 grams in 30 seconds (9.3 grams per minute). Second specimen, 8.65 grams in 60 seconds (8.65 grams per minute).

11.30 a.m. Injected 0.4 mgm, strychnine sulphate into jugular vein.

11.31 a.m. Reflexes exaggerated; no convulsions. Third specimen, 4.3 grams in 30 seconds (8.6 grams per minute). Fourth specimen, 8.8 grams in 60 seconds (8.8 grams per minute).

- 11.35 a.m. Blood pressure 114 mm. Hg.
 12.05 p.m. Blood pressure 96 mm. Hg. Fifth specimen, 4.35 grams in 30 seconds (8.7 grams per minute). Sixth specimen 7.95 grams in 60 seconds (7.95 grams per minute).
 12.15 p.m. Injected 0.5 mgm. strychnine into jugular vein. Marked clonic spasms.
 12.16 p.m. Seventh specimen, 2.8 grams in 30 seconds (5.6 grams per minute). Eighth specimen, 7.45 grams in 120 seconds (3.7 grams per minute).

During collection of the eighth specimen, tonic spasm occurred. Combined weight of adrenals 0.908 gram.

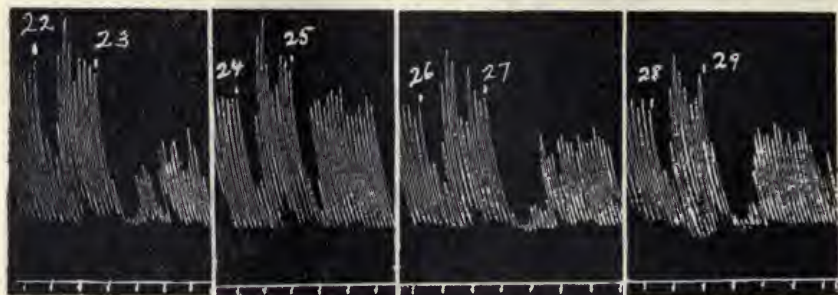


FIG. 4. INTESTINE TRACINGS. BLOODS FROM DOG 257

At 22, 24, 26, and 28 Ringer was replaced by indifferent (jugular) blood, and this at 23 by jugular blood made up with adrenalin to a concentration of 1:5,000,000; at 25 by jugular blood made up with adrenalin to a concentration of 1:8,300,000; at 27 by jugular blood made up with adrenalin to a concentration of 1:6,666,000; at 29 by the second adrenal specimen (collected before strychnine). All the bloods were diluted with three volumes Ringer, the adrenalin bloods after addition of the adrenalin. (Reduced to one-half.)

Tracings reproduced in figures 4 to 6 illustrate the assay of two of the adrenal specimens, the second, taken before the first injection of strychnine and the eighth, taken after a second strychnine injection. In figure 4 it is shown that the second specimen (observation 29) is much stronger than 1:8,000,000 (observation 25), decidedly weaker than 1:5,000,000 (observation 23), and not much different from 1:6,660,000 (observation 27).

Figure 5 indicates that the eighth specimen (observation 9) is much stronger than 1:3,300,000 (observaton 11), and figure 6 that it is stronger than 1:2,500,000 (observations 13 and 15), stronger than 1:1,660,000 (observations 15 and 17), and not very different from 1:830,000. It was confirmed by other observations (not reproduced) that the eighth specimen was decidedly stronger than 1:1,660,000, stronger than 1:1,250,000 and a little stronger than 1:830,000. Taking the second specimen at 1:6,700,000, we get 0.0013 mgm. per minute for the dog, or

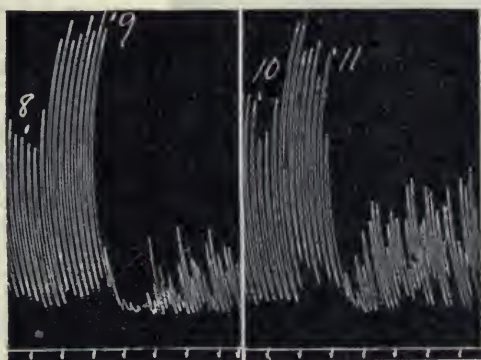


FIG. 5. INTESTINE TRACINGS. BLOODS FROM DOG 257

At 8 and 10 Ringer was replaced by indifferent (jugular) blood, and this at 9 by the eighth adrenal specimen (collected forty-five minutes after strychnine); and at 11 by jugular blood made up with adrenalin to a concentration of 1:3,300,000. The bloods were diluted with three volumes Ringer, the adrenalin blood after adding the adrenalin. (Reduced to one-half.)

0.0002 mgm. per kilogram per minute, the average output in dogs.

Taking the eighth specimen at 1:800,000, we get 0.0045 mgm. per minute for the dog, or 0.00075 mgm. per kilogram per minute. The output when the eighth specimen was collected was, therefore, nearly four times as great as before the first strychnine injection. The first dose of strychnine in this experiment only caused some exaggeration of the reflex excitability, but no convulsions.

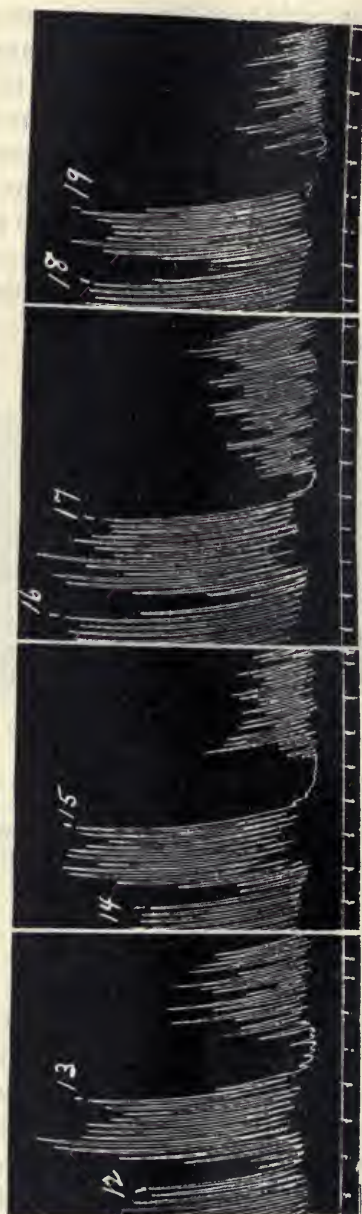


FIG. 6. INTESTINE TRACINGS. BLOODS FROM DOG 257

At 12, 14, 16 and 18 Ringer was replaced by indifferent (jugular) blood, and this at 13 by jugular blood made up with adrenalin to a concentration of 1:2,500,000; at 15 by the eighth adrenal blood specimen (collected forty-five minutes after strychnine); at 17 by jugular blood made up with adrenalin to a concentration of 1:1,660,000; and at 19 by jugular blood made up with adrenalin to a concentration of 1:830,000. All the bloods were diluted with three volumes Ringer, the adrenal in bloods after addition of the adrenalin. (Reduced to three-fifths.)

The fourth and sixth specimens did not show any definite increase in the output of epinephrin as compared with the second specimen. It was apparently after the second dose of strychnine, that the output was markedly increased.

In the next experiment (dog 246) a very large dose of strychnine was administered, causing convulsions almost immediately, and the epinephrin output was markedly increased in the specimen collected two minutes thereafter.

Condensed protocol; dog 246; female; weight, 7.5 kgm.

Anesthetized with morphine and ether. Obtained a specimen of indifferent blood from external jugular vein. Made cava pocket.

11.35 a.m. Started artificial respiration.

11.40 a.m. Blood pressure 110 mm. Hg. Collected adrenal blood.

First specimen, 8.8 grams in 30 seconds (17.6 grams per minute). Second specimen, 7.7 grams in 30 seconds (15.4 grams per minute).

11.52 a.m. Injected 2 mgm. strychnine into jugular vein. Powerful tetanic convulsions ensued almost immediately, and persisted throughout the collection of third and fourth specimens.

11.54 a.m. Third specimen, 7.3 grams in 30 seconds (14.6 grams per minute). Fourth specimen, 8.3 grams in 30 seconds (16.6 grams per minute).

12.00 m. Blood pressure 170 mm. Hg.

12.25 p.m. Tonic and clonic convulsions continued since collection of last adrenal specimen and dog died in convulsions at 12.28.

Combined weight of adrenals 1.26 grams.

The fourth specimen (collected after the strychnine injection) produced an enormously greater inhibition of the intestine segment than the second specimen (collected before strychnine was given) (fig. 7, observations 3 and 5). As the blood flow during collection of the fourth was somewhat greater than during collection of the second, the mere comparison of the curves is sufficient to show that the epinephrin output was increased. The assay proved that the second specimen was weaker than

1:10,000,000 adrenalin, weaker than 1:12,500,000, approximately the same as 1:15,000,000 (confirmed by several pairs of observations). Taking the second specimen at 1:15,000,000, we get 0.001 mgm. per minute for the dog, or 0.00013 mgm. per kilogram per minute. The fourth specimen was shown to be much stronger than 1:3,750,000 adrenalin, stronger than 1:2,500,000, stronger than 1:1,875,000; not very different from 1:1,250,000, probably somewhat weaker. Taking the fourth specimen even at 1:1,500,000, we get 0.011 mgm. per minute for the dog, or 0.0015

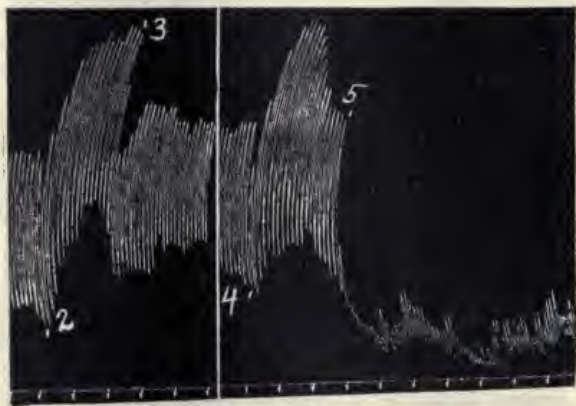


FIG. 7. INTESTINE TRACINGS. BLOODS FROM DOG 246

At 2 and 4 Ringer was replaced by indifferent (jugular) blood, and this at 3 by the second adrenal specimen (collected before strychnine); at 5 by the fourth adrenal specimen (collected three minutes after strychnine). The bloods were diluted with three volumes Ringer. (Reduced to one-half.)

mgm. per kilogram per minute. This is more than ten times the initial output.

It had been intended to wait for a considerable time before collecting a further specimen of adrenal blood, in order to see whether the increase in the epinephrin output produced by strychnine was transient or lasting. The animal died, however, in convulsions thirty minutes after the administration of the strychnine. No exact correspondence between the dose of strychnine and the increase in the epinephrin output could of course be expected. Something depends upon the initial rate

of output. If this is large it is scarcely likely that the relative increase produced by a given dose of strychnine will be as great as when the initial output is relatively low. There are, of course, other factors, among which the degree and nature of the anesthesia must be important, which influence the result. Thus, the largest dose given to dogs in the whole series of experiments was in dog 245. The dog died eventually in severe convulsions. The output of epinephrin in a specimen of adrenal blood collected four minutes after the injection of the strychnine was not quite twice as great as the relatively high output before the administration of the strychnine, in spite of the extreme excitation of the spinal motor mechanisms.

Condensed protocol; dog 245; male; weight, 9.5 kgm.

Anesthetized with morphine and ether. Obtained a specimen of indifferent blood from external jugular vein. Made cava pocket. Blood pressure 70 mm. Hg. Collected adrenal blood.

- 11.50 a.m. First specimen, 5.4 grams in 30 seconds (10.8 grams per minute). Second specimen, 9.5 grams in 60 seconds (9.5 grams per minute). Third specimen, 10 grams in 60 seconds (10 grams per minute). Started artificial respiration. Blood pressure 68 mm. Hg.
- 12.15 p.m. Injected 4.0 mgm. strychnine into jugular; tetanic convulsions occurred at once.
- 12.19 p.m. Fourth specimen, 4.25 grams in 16 seconds (16 grams per minute). Fifth specimen, 12.15 grams in 60 seconds (12.15 grams per minute).
- 12.25 p.m. Blood pressure 84 mm. Hg.
- 12.45 p.m. Dog died in severe convulsions.

Combined weight of adrenals 1.55 grams.

The third specimen (collected before strychnine) assayed at 1:3,500,000, the fifth (four minutes after strychnine) at 1:2,500,000. Figure 8 is reproduced to emphasize the point that while mere comparison of the inhibitory effects produced on the intestine segment by these two specimens (observation 3 and 5) is sufficient to show that the output of epinephrin must

have been increased in the fifth specimen, since the blood flow was somewhat greater in the fifth than in the third, no quantitative estimate of the difference can be made without a careful and detailed assay. The difference in concentration between the specimens was in reality much less than casual comparison of the curves might suggest.

In the next experiment (dog 248) a smaller, but still a large dose in proportion to the bodyweight was given, and specimens of adrenal blood were collected immediately (two and one-half minutes) after administration of the drug and forty minutes thereafter.

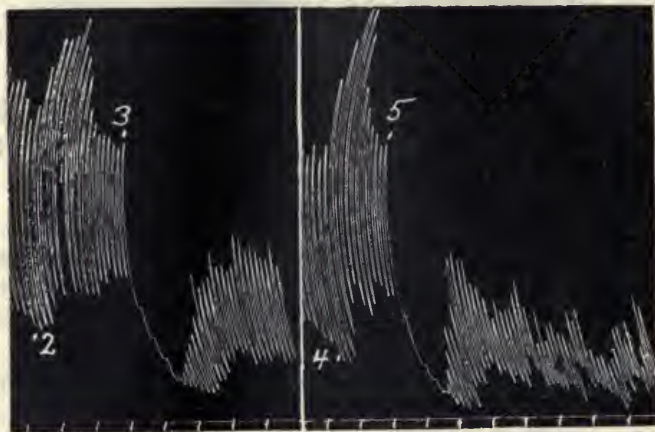


FIG. 8. INTESTINE TRACINGS. BLOODS FROM DOG 245

At 2 and 4 Ringer was replaced by indifferent (jugular) blood, and this at 3 by the third adrenal blood specimen (collected before strychnine); at 5 by the fifth adrenal specimen (collected five minutes after strychnine). The bloods were diluted with three volumes Ringer. (Reduced to one-half.)

Condensed protocol; dog 248; female; weight, 4.6 kgm.

Anesthetized with morphine and ether. Obtained a specimen of indifferent blood from the external jugular vein. Made cava pocket. Then collected adrenal blood. Blood pressure before collection of first adrenal specimen 58 mm. Hg.

12.00 m. First specimen, 5 grams in 30 seconds (10 grams per minute). Second specimen, 9.7 grams in 60 seconds (9.7 grams per minute).

- 12.15 p.m. Injected 0.5 mgm. strychnine into jugular vein. Severe tetanic convulsions occurred within one-half minute and artificial respiration was started at once.
- 12.17 p.m. Third specimen, 6.5 grams in 40 seconds (9.6 grams per minute). Fourth specimen, 8.9 grams in 60 seconds (8.9 grams per minute).
- 12.20 p.m. Blood pressure after collection of fourth specimen 125 mm. Hg.
- 12.26 p.m. Reflexes increased; convulsions ceased; spontaneous breathing.
- 12.40 p.m. Pulse 190 to 200 per minute; reflexes increased; spontaneous breathing.
- 12.50 p.m. Stopped artificial respiration.
- 12.55 p.m. Fifth specimen, 5.1 grams in 35 seconds (9.2 grams per minute). Sixth specimen, 8.15 grams in 60 seconds (8.15 grams per minute). Blood pressure after collection of sixth adrenal specimen 70 mm. Hg.

Obtained more jugular blood, and also arterial blood (from abdominal aorta). Combined weight of adrenals 0.8 gram.

The second specimen (taken before strychnine), the fourth (taken two and one-half minutes after strychnine) and the sixth (taken forty minutes after the administration of the drug) were assayed. As the blood flow differed little in the three specimens, even a comparison of their effects on the intestine segment (fig. 9) indicates that the output must have been greatly increased in the case of the fourth, and increased, although not so much in the case of the sixth specimen.

The assay showed that the second specimen was weaker than 1:10,000,000 adrenalin, weaker than 1:12,500,000, somewhat weaker than 1:15,000,000, and stronger than 1:18,750,000. It was confirmed by several observations that the concentration of the second specimen was less than 1:15,000,000 and probably not much greater than 1:18,750,000. Even if we take it at 1:16,000,000, we get for the epinephrin output before strychnine only 0.0006 mgm. per minute for the dog, or 0.00013 mgm. per kilogram per minute.

While the second specimen was assayed soon after the blood was collected, the fourth and sixth could only be provisionally

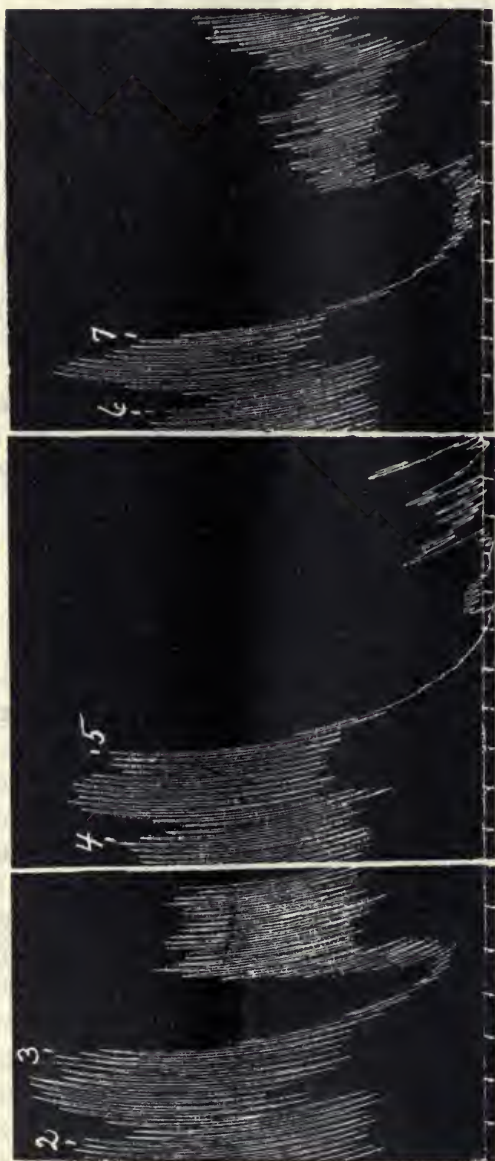


FIG. 9. INTESTINE TRACINGS. BLOODS FROM DOG 248

At 2, 4, and 6 Ringer was replaced by indifferent (jugular) blood, and this at 3 by the second adrenal specimen (collected before strychnine); at 5 by the fourth adrenal specimen (collected one minute after strychnine); at 7 by the sixth adrenal specimen (collected forty minutes after strychnine). The bloods were diluted with three volumes Ringer. (Reduced to three-fifths.)

assayed at that time. The assay was not completed till next day, the bloods being meanwhile kept on ice. Some epinephrin must, therefore, have been lost by these specimens, and the increase in the output is even greater than that yielded by the assay. The fourth specimen was much stronger than 1:3,750,000, stronger than 1:2,500,000 (confirmed by several observations), approximately the same as 1:1,250,000.

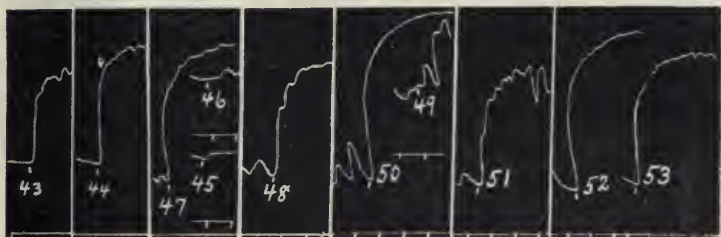


FIG. 10. UTERUS TRACINGS. BLOODS FROM DOG 248

At 43 Ringer was replaced by the sixth adrenal specimen (collected forty minutes after strychnine); at 44 by the fourth adrenal specimen (collected one minute after strychnine); at 45 by the second adrenal specimen (collected before strychnine); at 46 by arterial blood (collected after strychnine); at 47 by arterial blood made up with adrenalin to a concentration of 1:2,500,000; at 48 by arterial blood made up with adrenalin to a concentration of 1:3,750,000; at 49 by arterial blood made up with adrenalin to a concentration of 1:18,750,000; at 50 by the fourth adrenal specimen; at 51 by arterial blood made up with adrenalin to a concentration of 1:3,150,000; at 52 by the sixth adrenal blood specimen; and at 53 by arterial blood made up with adrenalin to a concentration of 1:3,150,000. All the bloods were diluted with one volume of Ringer, the adrenalin bloods after addition of the adrenalin. (Reduced to two-fifths.)

Taking the fourth specimen at 1:1,250,000, we get 0.007 mgm. per minute for the dog, or 0.0015 mgm. per kilogram per minute, fully ten times the initial output.

The sixth specimen was found to be much stronger than 1:5,000,000 adrenalin by intestine observations, and stronger than 1:3,125,000 (by uterus observations made next day, figure 10, observations 52 and 53, confirmed by observation 51). It was not as exactly assayed as the second and fourth specimens, but its concentration even twenty-four hours after collection indicated that the rate of output of epinephrin must have been

at least seven or eight times the initial output forty minutes after the administration of the strychnine. The conclusions drawn from the intestine assay were confirmed also for the other specimens by uterus observations (fig. 10).

The next experiment (dog 263) is an example of observations in which with a relatively small dose of strychnine, which caused only an increased reflex excitability unaccompanied at any time by convulsions, no increase in the output of epinephrin may be produced in the first specimen of adrenal blood drawn after the administration of the strychnine, but in later specimens a gradually increasing rate of output can be demonstrated; the increased output lasting for a considerable time, even when the exaggerated reflex excitability has disappeared.

Condensed protocol; dog 263; male; weight, 4.4 kgm.

Anesthetized with morphine and ether. Obtained a specimen of indifferent blood from femoral vein. Made cava pocket. Blood pressure 94 mm. Hg. Collected adrenal blood.

- 12.10 p.m. First specimen, 3.3 grams in 30 seconds (6.6 grams per minute). Second specimen, 6.15 grams in 69 seconds (6.15 grams per minute).
- 12.25 p.m. Injected 0.25 mgm. strychnine into jugular vein. Blood pressure 92 mm. Hg.—exaggerated reflexes.
- 12.26½ p.m. Third specimen, 3.35 grams in 30 seconds (6.7 grams per minute).
- 12.27 p.m. Fourth specimen, 5.7 grams in 60 seconds (5.7 grams per minute).
- 12.45 p.m. Blood pressure 88 mm. Hg. Fifth specimen, 3.5 grams in 30 seconds (7 grams per minute). Sixth specimen, 5.75 grams in 60 seconds (5.75 grams per minute).
- 12.50 p.m. Closed abdomen with clamps.
- 1.45 p.m. Blood pressure 76 mm. Hg.
- 1.49½ p.m. Seventh specimen, 2.2 grams in 30 seconds (4.4 grams per minute).
- 1.50 p.m. Eighth specimen, 7.4 grams in 120 seconds (3.7 grams per minute).

Obtained arterial blood from abdominal aorta. Combined weight of adrenals 0.78 gram.

In this animal the initial output before the strychnine injection was rather high. The second specimen was shown to be stronger than 1:4,300,000 adrenalin; decidedly weaker than 1:2,500,000 and a little weaker than 1:3,500,000. It was finally taken as equal to 1:3,800,000, giving 0.0016 mgm. per minute for the animal, or 0.00035 mgm. per kilogram per minute. The fourth specimen (collected two minutes after the strychnine injection) was somewhat weaker than the second, although the blood flow was rather less. It was assayed at 1:4,300,000, corresponding to an output per minute for the dog of 0.0013 mgm., or 0.0003 mgm. per kilogram per minute. We cannot always be certain of a difference as small as this, but in this case there seems no doubt from the sharpness of the assay, and from the fact that qualitatively the fourth specimen gave a smaller reaction than the second, in spite of the smaller blood flow during collection of the former, that the output was somewhat diminished in the first stage of the strychnine action. If this were an isolated observation we should not think of basing such a conclusion upon it. But we have seen in other experiments much more striking instances of a transient preliminary diminution in the epinephrin output after the administration of strychnine. It is to be expected that if such a phenomenon exists, it will be more easy to detect it when small doses of the drug are used, or where it is administered subcutaneously instead of intravenously and where accordingly the absorption is slower, and the transient inhibitory action of the drug is not masked by a rapidly developed, large and lasting augmentation of the rate of output. It is precisely in these circumstances that the evidence of the preliminary diminution in the output is clearest. Instances of this will be given in the proper place.

The sixth specimen (collected ten and one-half minutes after the strychnine injection) showed a definite augmentation of the epinephrin output. It was assayed at 1:2,800,000, corresponding to an output for the dog of 0.002 mgm. per minute, or 0.00045 mgm. per kilogram per minute. The eighth specimen (obtained eighty-five minutes after administration of the strychnine) was found to be stronger than 1:1,400,000 adrenalin,

slightly weaker than 1:1,000,000. It was taken as equal to 1:1,100,000, corresponding to an output of 0.0034 mgm. per minute for the animal, or 0.00075 mgm. per kilogram per minute, fully double the initial output. The percentage of serum in the eighth specimen was 66.5 determined by the electrical method, and 65 per cent by the haematocrit with prolonged centrifugalisation. The concentration of epinephrin in the serum was therefore 1:730,000.

Having demonstrated in the experiments on dogs, with intravenous injection of strychnine, the effects produced on the rate of epinephrin output by large and small doses, some experiments were next made on the effects of the drug when administered subcutaneously. We began with a large (convulsant) dose in order to see at once whether the general course of the action was the same.

This proved to be the case, the most notable point of difference being the clearer indication of a transient preliminary stage of inhibition or reduction in the rate of liberation of epinephrin.

Condensed protocol; dog 306; male; weight, 5.05 kgm.

Anesthetized with morphine and ether. Obtained indifferent blood from the jugular vein. Made cava pocket. Then collected adrenal blood.

- 11.30½ a.m. First specimen, 3.6 grams in 30 seconds (7.2 grams per minute).
- 11.31 a.m. Second specimen, 8.2 grams in 60 seconds (8.2 grams per minute). Blood pressure 84 mm. Hg.
- 11.37½ a.m. Injected 1.0 mgm. strychnine hypodermically.
- 11.40 a.m. Injected 1.0 mgm. strychnine hypodermically.
- 11.44 a.m. Tonic convulsions—started artificial respiration, which was continued for the rest of the experiment.
- 11.44½ a.m. Third specimen, 4 grams in 30 seconds (8 grams per minute).
- 11.45 a.m. Fourth specimen, 7.8 grams in 60 seconds (7.8 grams per minute). Blood pressure 115 mm. Hg.
- 11.50 a.m. Fifth specimen, 16.1 grams in 60 seconds (16.1 grams per minute). Blood pressure 110 mm. Hg.

- 11.58 a.m. Clonic convulsions—spontaneous breathing.
12.09 p.m. Sixth specimen, 6.3 grams in 30 seconds (12.6 grams per minute).
12.09½ p.m. Seventh specimen, 11.1 grams in 60 seconds (11.1 grams per minute).
12.10½ p.m. Eighth specimen, 9.55 grams in 60 seconds (9.55 grams per minute). Blood pressure 60 mm. Hg.
12.30 p.m. Ninth specimen, 3.35 grams in 30 seconds (6.7 grams per minute).
12.30½ p.m. Tenth specimen, 7.55 grams in 60 seconds (7.55 grams per minute). Clonic convulsions still present; spontaneous breathing. Blood pressure 50 mm. Hg.

Abdominal aorta blood obtained. Combined weight of adrenals 0.705 gram.

The maximum increase was as large as in any of the experiments with intravenous injection (up to ten times the initial output). More than half an hour after injection of the strychnine the output was still eight times as great as before the drug was administered. Fifty minutes after the injection the output was still distinctly increased. Specimens of the tracings are given in figures 11 to 15. In figure 11 it is demonstrated that the fourth adrenal specimen, collected five minutes after the last injection of strychnine, while strong tonic convulsions were going on, caused no inhibition of the intestine segment, while the second specimen, collected before injection of strychnine caused a distinct effect. The difference was considerably greater in other observations when the segment had become more sensitive. Since the blood flows during collection of the second and fourth specimens were practically equal, a little less indeed, for the fourth, this is of itself sufficient to show that the output at the time of collection of the fourth specimen was diminished. Comparison of the effect produced by the fourth with that produced by the fifth specimen (fig. 12) indicates a very great increase in the output in the five minutes following the collection of the fourth specimen.

The epinephrin assay showed that the second specimen (collected before strychnine was given) was decidedly weaker than 1:5,000,000 adrenalin, weaker than 1:6,500,000, distinctly

weaker than 1:7,000,000, and somewhat weaker than 1:7,850,000 (confirmed by three separate pairs of observations). It was stronger than 1:10,000,000, and approximately equal to 1:8,500,000 (fig. 13), corresponding to an output of 0.001 mgm. per minute for the dog, or 0.0002 mgm. per kilogram per minute.

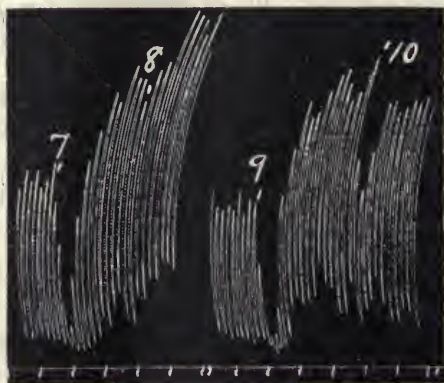


FIG. 11. INTESTINE TRACINGS. BLOODS FROM DOG 306

At 7 and 9 Ringer was replaced by arterial blood, and this at 8 by the fourth adrenal specimen; at 10 by arterial blood made up with adrenalin to a concentration of 1:15,000,000. The bloods were diluted with one volume Ringer, the adrenalin blood after addition of the adrenalin. (Reduced to one-half.)

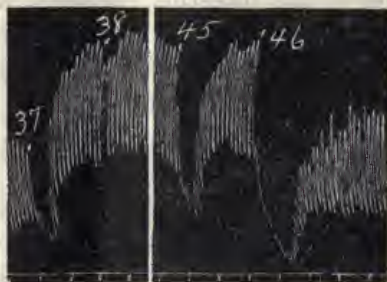


FIG. 12. INTESTINE TRACINGS. BLOODS FROM DOG 306

At 37 and 45 Ringer was replaced by indifferent (arterial) blood (collected after strychnine); at 38 by the fourth adrenal specimen (collected eight minutes after injection of strychnine) at 46 by the fifth adrenal specimen (collected ten minutes after strychnine). The bloods were diluted with three volumes Ringer. (Reduced to one-half.)

It was shown that 1:30,000,000 adrenalin gave a distinct inhibition at a time when the fourth specimen caused practically no effect. The fourth specimen must, therefore, have been weaker than 1:30,000,000. It was not proved that it contained

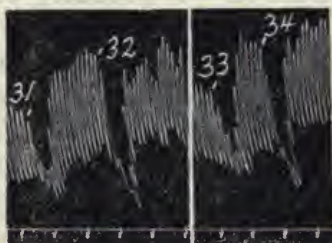


FIG. 13. INTESTINE TRACINGS. BLOODS FROM DOG 306

At 31 and 33 Ringer was replaced by indifferent (jugular) blood, and this at 32 by jugular blood with adrenalin added to make a concentration of 1:3,500,000; at 34 by the second adrenal specimen (collected before strychnine). The bloods were diluted with three volumes Ringer, the adrenalin blood after adding the adrenalin. (Reduced to one-half.)

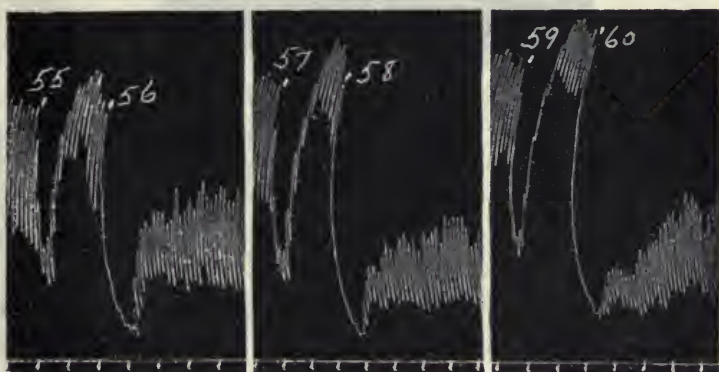


FIG. 14. INTESTINE TRACINGS. BLOODS FROM DOG 306

At 55, 57 and 59 Ringer was replaced by arterial blood (collected after strychnine); at 58 the eighth adrenal specimen (collected thirty minutes after subcutaneous injection of strychnine) replaced the arterial blood; at 56 and 60 the arterial blood was replaced by arterial blood made up with adrenalin to a concentration of 1:1,400,000 and 1:1,000,000 respectively. All the bloods were diluted with three volumes Ringer, the adrenalin bloods after addition of the adrenalin. (Reduced to one-half.)

any epinephrin. The output at the time of collection of this specimen was certainly less than 0.00025 mgm. per minute for the animal, or 0.00005 mgm. per kilogram per minute, i.e., less than one-fourth the initial output before administration of strychnine. The reduction was probably considerably greater than this.

The fifth specimen, collected ten minutes after the strychnine injection, in spite of the greatly increased blood flow had a much greater concentration of epinephrin than the second. It was found to be stronger than 1:2,250,000 adrenalin, somewhat weaker than 1:1,500,000. It was taken at 1:1,600,000, corresponding to an output of 0.01 mgm. per minute for the dog,



FIG. 15. UTERUS TRACINGS. BLOODS FROM DOG 306

At 58, Ringer was replaced by jugular blood; at 59 by the tenth specimen; at 60 by the fifth specimen; at 61 by the eighth specimen. All the bloods were diluted with five volumes Ringer. (Reduced to one-half.)

or 0.002 mgm. per kilogram per minute; ten times the initial output.

The eighth specimen, collected half an hour after injection, was found to be decidedly stronger than 1:3,000,000 adrenalin, stronger than 1:1,400,000, probably not quite as strong as 1:1,000,000, but not much different from it (fig. 14). It was taken at 1:1,200,000, corresponding to an output of 0.008 mgm. per minute for the dog, or 0.0016 mgm. per kilogram per minute; eight times the initial output. The proportion of serum in the blood was 46.6 per cent as determined by the electrical method. The haematocrit, even after thirty-two and one-half minutes, gave only 33 per cent, although the proportion was slowly mount-

ing as the centrifugalization went on. The haematocrit method is quite unsuitable when the proportion of serum is small and the corpuscles separate slowly. The concentration of epinephrin in the serum of the eighth specimen, taking the percentage at 46.5, was 1:560,000, very nearly the possible normal maximum. Since this maximum does not seem to be altered by strychnine, although as will be shown in another paper it may be markedly affected by other drugs, the calculated output for the eighth specimen might have been even greater had there been at this time a larger blood flow to carry off the epinephrin.

The tenth specimen, collected fifty minutes after the administration of strychnine, could not be satisfactorily assayed owing to a mistake, but it was shown that its concentration was such as to leave no doubt that at this time the epinephrin output was still decidedly increased.

The uterus tracings reproduced in figure 15 confirm the intestine observations and show that the inhibitory reactions of the intestine segments were due to epinephrin. The fourth specimen gave with the uterus a very small reaction compared with the other specimens.

In the next experiment, the last on dogs to be cited, a much smaller quantity of strychnine (two doses amounting to 0.09 mgm. per kilogram of bodyweight in all) injected subcutaneously was found to cause a distinctly increased output of epinephrin. The dose was below that required to produce any noticeable effect on the reflex excitability. The blood pressure was not altered nor would it have been possible for anyone studying the behavior of the animal to conclude that strychnine had been administered.

Condensed protocol; dog 309; female; weight, 8.1 kgm.

Anesthetized with morphine and ether. Obtained a specimen of indifferent blood from the jugular vein. Made cava pocket. Then collected adrenal blood.

11.00 a.m. Blood pressure 108 mm. Hg.

11.01½ a.m. First specimen, 7.25 grams in 30 seconds (14.5 grams per minute).

- 11.02 a.m. Second specimen, 15.9 grams in 60 seconds (15.9 grams per minute).
11.09 a.m. Injected 0.5 mgm. strychnine hypodermically.
11.12 a.m. Reflexes not increased.
11.15 a.m. Injected 0.25 mgm. strychnine hypodermically.
11.19 a.m. No noticeable increase of reflexes.
11.21 a.m. Third specimen, 6.8 grams in 30 seconds (13.6 grams per minute).
11.21½ a.m. Fourth specimen, 13.6 grams in 60 seconds (13.6 grams per minute).
11.22½ a.m. Blood pressure 100 mm. Hg.
11.25 a.m. Reflexes not demonstrably increased; administered a few whiffs of ether.
11.37 a.m. Fifth specimen, 5.2 grams in 30 seconds (10.4 grams per minute).
11.37½ a.m. Sixth specimen, 11.1 grams in 60 seconds (11.1 grams per minute).
11.38½ a.m. Blood pressure 88 mm. Hg.
12.07 p.m. Seventh specimen, 4.2 grams in 30 seconds (8.4 grams per minute).
12.07½ p.m. Eighth specimen, 10.4 grams in 60 seconds (10.4 grams per minute).
12.08½ p.m. Blood pressure 85 mm. Hg.

No distinctly increased reflex excitability was present at any time. Another specimen of indifferent blood was obtained from the right heart while the animal was still in good condition. Combined weight of adrenals 0.942 gram.

The epinephrin assay showed that the second adrenal specimen (collected before strychnine) was stronger than the fourth specimen (collected six and one-half minutes after the last dose of strychnine), although the blood flow was somewhat greater for the second than for the fourth (fig. 16, observations 2 and 4). This is a further indication that a transient diminution of the output of epinephrin may precede the increase, although, of course, if this was an isolated observation no weight would be attached to such a relatively small difference.

The sixth adrenal specimen (taken more than twenty-two minutes after the last dose of strychnine) was distinctly stronger

than the second (fig. 16, observation 6). The assay showed that the second specimen was much stronger than 1:10,000,000 adrenalin, weaker than 1:5,000,000 (confirmed by several observations), stronger than 1:7,100,000, and approximately the same as 1:6,100,000, corresponding to an output of 0.0026 mgm. per minute for the dog, or 0.0003 mgm. per kilogram per minute.

The sixth specimen was found to be stronger than 1:3,500,000, stronger than 1:3,000,000 (confirmed by several observations), somewhat weaker than 1:2,100,000 (fig. 17, observations 60 and 62, confirmed by other observations). It was finally taken at

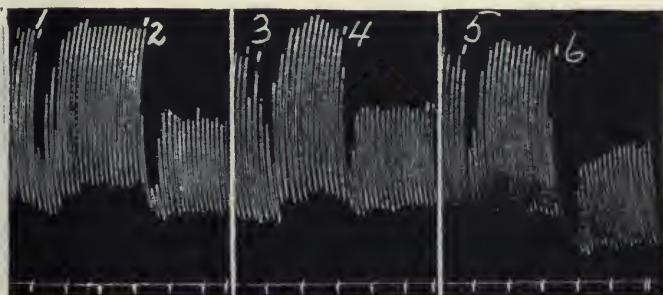


FIG. 16. INTESTINE TRACINGS. BLOODS FROM DOG 309

At 1, 3 and 5 Ringer was replaced by jugular blood, and this at 2 by the second adrenal specimen (collected before injection of strychnine); at 4 by the fourth adrenal specimen (collected six minutes after subcutaneous injection of strychnine), at 6 by the sixth adrenal specimen (collected twenty-five minutes after injection of strychnine). All the bloods were diluted with three volumes Ringer. (Reduced to one-half.)

1:2,300,000, corresponding to an output of 0.0048 mgm. per minute for the dog, or 0.0006 mgm. per kilogram per minute. At this stage, therefore, the initial output was doubled.

The eighth specimen (collected about an hour after the first dose of strychnine) was shown to be much stronger than 1:3,000,000, much stronger than 1:2,500,000, stronger than 1:2,100,000 (fig. 17, observations 62 and 64), and weaker than 1:1,800,000 (fig. 17, observations 64 and 66). It was confirmed by the uterus that the eighth was the strongest of all the specimens. It was finally taken at 1:2,000,000, corresponding to an output of 0.005 mgm. per minute for the dog, or 0.0006 mgm.

per kilogram per minute. The increase in the output was at least as great as half an hour earlier. It is not known whether the crest of the increase might not have been reached somewhere between the sixth and eighth specimens, in which case the maximum increase would have been greater than that found in either specimen.

Corroborative evidence that strychnine increases the output of epinephrin was obtained by studying the effect upon the blood pressure of adrenal vein blood collected in a cava pocket for a definite time, before and after administration of strychnine and



FIG. 17. INTESTINE TRACINGS. BLOODS FROM DOG 309

At 59, 61, 63, and 65 Ringer was replaced by jugular blood, and this at 60 by the sixth adrenal specimen (collected twenty-five minutes after injection of strychnine); at 62 by jugular blood to which was added adrenalin to make a concentration of 1:2,100,000; at 64 by the eighth adrenal specimen (collected fifty minutes after injection of strychnine); at 66 by jugular blood to which was added adrenalin to make a concentration of 1:1,800,000. All the bloods were diluted with three volumes Ringer, the adrenalin bloods after adding the adrenalin. (Reduced to one-half.)

then released into the circulation. It is not easy, with large doses, to overcome the difficulty caused by the great rise of blood pressure produced by the strychnine itself and which is not eliminated by curare. But we succeeded in getting a sufficient number of satisfactory tracings to demonstrate by this method also that strychnine produces a definite increase in epinephrin output. One experiment (on dog 278) is cited as an example, with specimens of the blood pressure tracings (figs. 18 to 21). The animal, a bitch weighing 5.6 kilograms, was anesthetized with morphine

and ether. A "long" cava pocket was formed in the usual way, the abdominal aorta, but not the intestinal arteries being tied. A blood pressure tracing was taken from a carotid. The vagi were cut. Numerous pocket experiments were made to determine the output of epinephrin and the effect of strychnine upon it. In figure 18, before the administration of strychnine, the pocket was closed at 2, opened after ninety seconds at 3. The mean arterial pressure before the opening of the pocket was 84 mm. of mercury, the maximum after opening 106 mm. 0.5 mgm. of strychnine sulphate was injected into the jugular two to three minutes before 12 (fig. 18) in two doses. Convulsions ensued, but

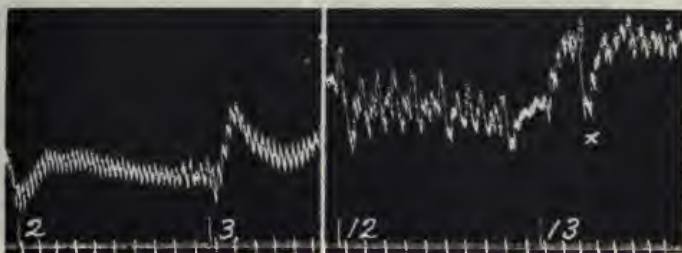


FIG. 18. BLOOD PRESSURE TRACINGS FROM DOG 278

Cava pocket closed at 2, opened after ninety seconds at 3 (before strychnine); cava pocket closed at 12, opened after ninety seconds at 13 (four minutes after strychnine injection). The abrupt descent in the curve marked by a cross coincided with a convulsion. The signal marks on the time trace are 2 mm. to the right of the corresponding points on the blood pressure curve. Time in ten-second intervals. Line of zero pressure (same as time trace) moved up 27 mm. and the figure then reduced to two-thirds.

the natural breathing continued to be sufficient. At 12 (four minutes after the first dose of strychnine) the cava pocket was closed, at 13 it was opened after ninety seconds. The mean pressure before the opening of the pocket was 105 mm. of mercury and the maximum pressure after it was released 140 mm. Obviously the adrenal blood collected in the pocket after strychnine, caused a decidedly greater effect on the blood pressure than the blood collected for the same time before strychnine. Not only was the rise of pressure after strychnine greater, but it was much more sustained, the maximum level being maintained for some

minutes after 13. An approximate estimate of the rate of output of epinephrin before and after strychnine (fig. 19) showed also a definite increase after strychnine. The pocket was closed at 5 (before strychnine), and opened at 7 after two minutes. The effect produced on the blood pressure at 7 is manifestly less than that due to injection into the jugular at 6 of 0.5 cc. of a 1:300,000 solution of adrenalin. Nine minutes after the strychnine injection a corresponding pocket experiment (14 to 16) was made. Although the time of closure of the pocket was only ninety seconds, the rise of pressure following release of the pocket was

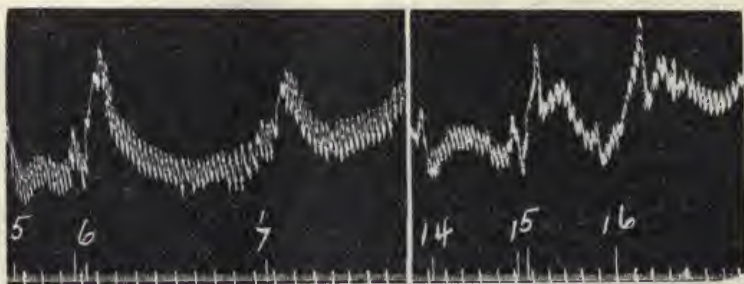


FIG. 19. BLOOD PRESSURE TRACINGS FROM DOG 278

Cava pocket closed at 5 (before strychnine injection), opened after two minutes at 7. At 6, 0.5 cc. of 1:300,000 adrenalin injected into jugular. Cava pocket (nine minutes after strychnine injection) closed at 14, opened after ninety seconds at 16. At 15, 0.5 cc. of 1:300,000 adrenalin injected into jugular. The signal marks on the time trace are 2 mm. to the right of the corresponding points on the blood pressure curve, Line of zero pressure (same as time trace) moved up 33 mm. and the figure then reduced to two-thirds.

distinctly greater and more sustained than that due to injection of 0.5 cc. of 1:300,000 adrenalin.

In both cases the adrenalin was injected while the pocket was closed, as we have seen some evidence that the effect of a given quantity of adrenalin injected into the circulation, may not be quite the same when the normal epinephrin discharge into the blood is going on, as when the adrenal blood is prevented from escaping. Any evidence that a given amount of adrenalin affects the blood pressure differently according to whether the normal epinephrin discharge is occurring or not is at the same time evi-

dence that this normal discharge is exerting some influence upon the circulation. While we have not made any special experiments on this point and have only incidental observations to go upon, it is obvious that if the normal amount of epinephrin continuously liberated from the adrenals is already affecting the circulatory mechanism to a sensible extent, the change produced by the addition of a given amount of adrenalin may be less than when the same amount of adrenalin is thrown in at a time when epinephrin has disappeared from the blood stream. In the one case the increase in circulation will be from zero, in the other from a small, but still positive amount. This factor would naturally be still more important when the epinephrin output under the influence of strychnine was proceeding at an accelerated rate. Be this as it may, it is clear that the conditions are more nearly similar when we compare the action of a given dose of adrenalin injected while the pocket is closed off and, therefore, into a circulation bare of epinephrin with the effect of adrenal blood released from the pocket, than when the adrenalin is injected with the normal epinephrin discharge proceeding. It will be seen from the curves that there is plenty of time with the duration of pockets employed, for the adrenalin reaction to have passed off before the pocket is released. Complete equality in the conditions could be attained by clamping off the pocket after it was emptied, so as to ensure that the blood pressure reaction caused by the contents of the pocket is not reinforced by the succeeding steady discharge.

The epinephrin output before administration of strychnine, was approximately estimated by auto-assay at 0.0006 mgm. per minute for the dog, or 0.0001 mgm. per kilogram per minute. Ten minutes after injection of strychnine an approximate assay gave 0.0013 mgm. per minute for the dog, or 0.00023 mgm. per kilogram per minute. The assays were rather rough as it was necessary to estimate rapidly the increasing output as the strychnine action developed, and at best, blood pressure assays cannot be as delicate as those made with rabbit segments. Nevertheless, it is certain that the output of epinephrin was at least doubled in the first ten minutes after the strychnine was given.

Thirty minutes after injection of strychnine the rise of pressure produced by releasing a pocket which had been closed for ninety seconds was much greater than that caused by injection of 0.5 cc. of 1:150,000 adrenalin and decidedly less than that caused by 0.5 cc. of 1:75,000 adrenalin (fig. 20).

The output at this time was at least 0.0025 mgm. per minute for the dog, or 0.00045 mgm. per kilogram per minute; fully four times the output before strychnine. The adrenalin injections at this point were made with the pocket open. Several additional pocket experiments and injections of adrenalin with the pocket open and closed confirmed the conclusion that strychnine

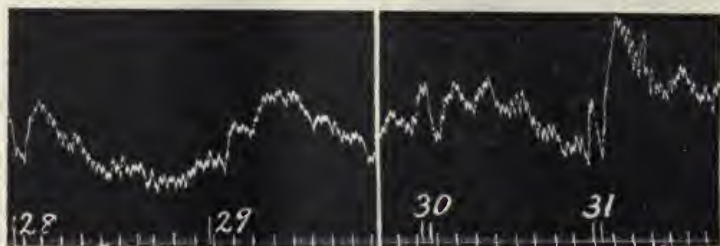


FIG. 20. BLOOD PRESSURE TRACINGS FROM DOG 278

Cava pocket (thirty-four minutes after strychnine injection) closed at 28, opened after ninety seconds at 29. At 30, 0.5 cc. of 1:150,000 adrenalin; at 31, 0.5 cc. of 1:75,000 adrenalin injected into jugular. Time in ten second intervals. The signal marks on the time trace are 2 mm. to the right of the corresponding points on the blood pressure curve. Line of zero pressure (same as time trace) moved up 27 mm. and the figure then reduced to two-thirds.

had caused a decided increase in the rate of epinephrin output and that the increase was greater forty to fifty minutes after the strychnine injection than ten to fifteen minutes after it. Curare (2 cc. of a 1 per cent solution) had been administered intravenously in the interval between the tracings shown in figures 19 and 20. Paralysis was complete in ten to fifteen minutes and artificial respiration was maintained thereafter. That some of the bulbo-spinal centres were still extremely sensitive to strychnine was shown by the effect of the intravenous injection of 0.5 mgm. forty minutes after the administration of curare and fourteen minutes after the last observation reproduced in figure 20.

Twenty seconds after injection of the strychnine the blood pressure rose almost vertically from 100 mm. to 230 mm. of mercury. The rise was not accompanied by any muscular contraction and was long maintained. After twelve minutes the pressure was still 200 mm. Preparations were now made to expose the cervical cord. There was sharp hemorrhage and the pressure fell to 110 mm. before the canal was opened. On sectioning the cord the pressure fell to 80 mm., then to 40 mm. and finally to 10 mm.

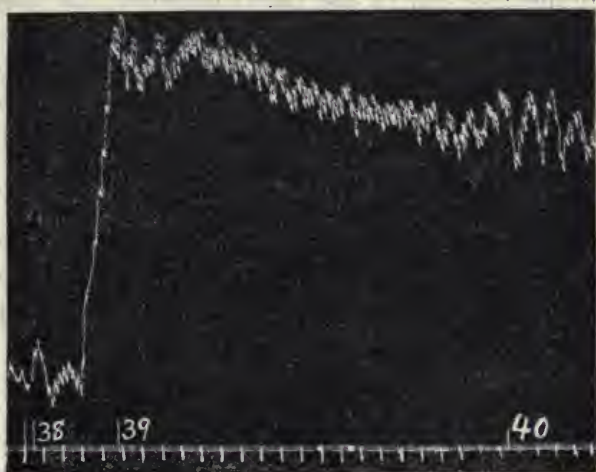


FIG. 21. BLOOD PRESSURE TRACINGS FROM DOG 278

At 38, 0.5 mgm. strychnine was injected intravenously; at 39 the cava pocket was closed and at 40 it was opened; duration of the pocket collection was three minutes. Time trace ten second intervals. Line of zero pressure moved up 35 mm. and the figure then reduced to two-thirds.

and to zero. Increased liberation of epinephrin might have been a minor factor in the preceding rise of pressure. A gradual fall of pressure of 25 mm. of mercury was produced by closing off the cava pocket at the moment when the rise had reached its maximum (fig. 21). We desire to point out clearly that even if no effect were produced upon the pressure by shutting off the adrenal blood, the epinephrin might still have been taking an appreciable share in initiating and sustaining the rise of blood pressure. For the activity of the vasomotor center was obviously

so intense that the withdrawal of the epinephrin from the blood might be easily and immediately compensated for by the nervous mechanism, all the more as it is, of course, impossible by suddenly shutting off the adrenal blood to cause an *instantaneous* disappearance of epinephrin from the circulation. The possibility that vasomotor compensation may mask the epinephrin effect has not been sufficiently considered by writers who have argued that because the clipping of the adrenal veins does not cause an immediate fall of the blood pressure, the normal epinephrin discharge cannot have been exerting any influence whatever upon the pressure. We do not suggest that any of the sympathico-mimetic actions of the naturally secreted epinephrin can ever under ordinary conditions rival in magnitude the actions of the nervous mechanisms; the epinephrin factor must be considered at best a subordinate one. But when on the strength of a supposedly crucial experiment it is denied that epinephrin enters even in the slightest degree into a given physiological reaction, it must be carefully considered whether the experiment is really crucial.

EXPERIMENTS ON CATS

In the cats, as in the dogs, we began with large doses. In the first experiments urethane (1.5 to 1.75 gram per kilogram by stomach tube) was used as the anesthetic. Later on evidence was obtained that urethane is not the most suitable anaesthetic when it is desired to elicit the maximal effect of a given dose of strychnine, owing to its action in diminishing the reflex excitability of the cord. Qualitatively, however, these experiments yielded the same result as those with other anaesthetics.

Condensed protocol; cat 228; female; weight, 2.5 kgm.

Anesthetized with urethane. Obtained a specimen of indifferent blood from the jugular vein. Made cava pocket. Started artificial respiration (good spontaneous respiration). Then collected adrenal blood.

10.45 a.m. First specimen, 2.1 grams in 30 seconds (4.2 grams per minute). Second specimen, 5.2 grams in 90 seconds (3.5 grams per minute).

- 11.00 a.m. Injected 1 mgm. strychnine into jugular vein. Tonic convulsions occurred in about 1 minute.
- 11.02 a.m. Third specimen, 2.15 grams in 30 seconds (4.3 grams per minute). Fourth specimen, 6.7 grams in 120 seconds (3.3 grams per minute).
- 11.05 a.m. Closed abdomen with clamps.
- 11.30 a.m. Reflexes only slightly increased.
- 11.45 a.m. Reflexes not exaggerated. Fifth specimen, 2.4 grams in 30 seconds (4.8 grams per minute). Sixth specimen, 6.75 grams in 120 seconds (3.25 grams per minute).

Obtained another specimen of jugular blood. Combined weight of adrenals 0.492 gram.

The observations on both intestine and uterus segments permitted a good assay. As so many intestine tracings have been already reproduced, only some specimens of the uterus tracings are given in figure 22. With the intestine it was shown that the second specimen (collected before strychnine) was somewhat weaker than 1:5,300,000 adrenalin, and somewhat stronger than 1:6,300,000. The concentration was taken at 1:5,800,000, corresponding to an output of 0.0006 mgm. per minute for the cat, or 0.00024 mgm. per kilogram per minute.

The fourth specimen (collected two and one-half minutes after injection of the strychnine) was found to be stronger than the second. It was assayed at 1:3,300,000. Figure 22 (observations 66 and 68) indicates that it was stronger than 1:3,500,000. The output of epinephrin, taking the fourth specimen at 1:3,300,000 is 0.001 mgm. per minute for the cat, or 0.0004 mgm. per kilogram per minute, i.e., nearly twice as much as the original output.

The sixth specimen (collected forty-five minutes after injection of strychnine) was found both with intestine and uterus to be stronger than the fourth (fig. 22, observations 65 and 66). In observations 50 and 51, the fourth and sixth specimens produced about the same effect upon the uterus segment, but this is because the dilution employed for these two observations was such that even the fourth specimen caused a practically maximal effect. The sixth specimen was assayed at 1:1,650,000

adrenalin, corresponding to an output of 0.002 mgm. per minute for the cat, or 0.0008 mgm. per kilogram per minute, between three and four times the initial output.

In the next experiment a cat of exactly the same weight received the same dose of strychnine as cat 228. It was planned to collect the last specimen at a much longer interval from the strychnine injection, but on account of the deterioration of the circulation it had to be taken in one hour.

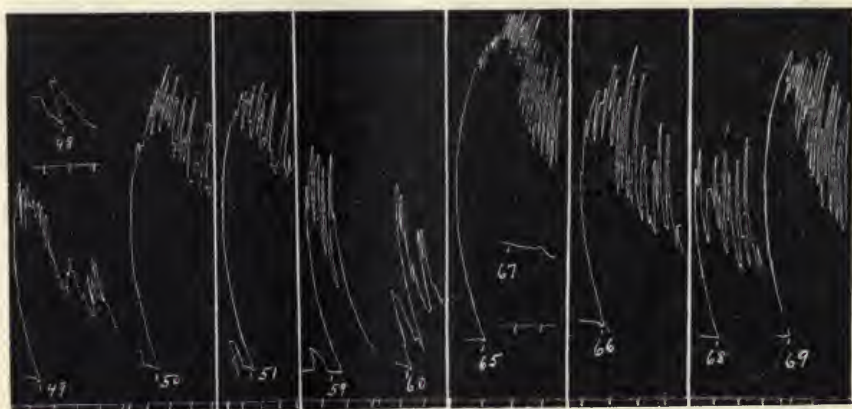


FIG. 22. UTERUS TRACINGS. BLOODS FROM CAT 228

At 48 Ringer was replaced by jugular blood (collected after strychnine injection); at 49 by the second adrenal specimen (collected before strychnine); at 50 by the fourth adrenal specimen (collected immediately after strychnine); at 51 by the sixth adrenal specimen (collected forty-five minutes after strychnine). In observations 48-51 the bloods were diluted with ten volumes Ringer. At 59 Ringer was replaced by the fourth; at 60 by the sixth adrenal specimen (both bloods being diluted with nineteen volumes Ringer); at 65 Ringer was replaced by the sixth; at 66 by the fourth adrenal specimen; at 67 by jugular blood (collected after strychnine); at 68 by the jugular blood made up with adrenalin to a concentration of 1:3,500,000; at 69 by the jugular blood made up with adrenalin to a concentration of 1:1,750,000. In observations 65 to 69 the bloods were diluted with four volumes Ringer, the adrenalin bloods after adding the adrenalin. (Reduced to two-fifths.)

Condensed protocol; cat 239; male; weight, 2.41 kgm.

Anesthetized with urethane. Obtained a specimen of indifferent blood from jugular vein. Made cava pocket. Then collected adrenal blood.

- 11.05 a.m. First specimen, 1.95 grams in 45 seconds (2.6 grams per minute). Second specimen, 4.6 grams in 120 seconds (2.3 grams per minute).
- 11.15 a.m. Blood pressure 87 mm. Hg.
- 11.16 a.m. Started artificial respiration (breathing well, spontaneously).
- 11.19 a.m. Injected 1 mgm. strychnine into jugular vein. Reflexes exaggerated at once, opisthotonos.
- 11.20 a.m. Blood pressure 64 mm. Hg.
- 11.22 a.m. Third specimen, 2 grams in 60 seconds (2 grams per minute). Fourth specimen, 3.5 grams in 120 seconds (1.75 grams per minute).
- 11.27 a.m. Blood pressure 72 mm. Hg. Reflexes increased.
- 11.30 a.m. Closed abdomen with clamps.
- 12.15 p.m. Blood pressure 56 mm. Hg.
- 12.18 p.m. Fifth specimen, 0.75 gram in 40 seconds (1 gram per minute). Sixth specimen, 3.05 grams in 240 seconds (0.76 gram per minute).

Obtained more jugular blood; also arterial (abdominal aorta) blood. Combined weight of adrenals 0.35 gram.

The second specimen (collected before strychnine) produced a much smaller effect on the intestine segment than the fourth (taken four minutes after injection), and the fourth, a much smaller effect than the sixth (taken one hour after the strychnine injection). Typical tracings are reproduced in figure 23. The technical point must be again emphasised, that from the mere inspection of these tracings no conclusion whatever can be drawn as to any increase in the epinephrin output, since the flow was smaller for the fourth than for the second specimen, and much smaller for the sixth than for the fourth. A careful assay was required to determine that at the time of collection of the fourth specimen the output of epinephrin was definitely increased, although only to a small extent, while at the time of collection of the sixth specimen it was more than doubled.

The second specimen was assayed at 1: 5,000,000, corresponding to an output per minute of 0.00046 mgm. epinephrin per minute for the cat, or 0.0002 mgm. per kilogram per minute.



FIG. 23. INTESTINE TRACINGS. BLOODS FROM CAT 239

At 8, 10 and 16 Ringer was replaced by indifferent (jugular) blood, and this at 9 by the second adrenal specimen (collected before strychnine); at 11 by the fourth adrenal specimen (collected four minutes after strychnine); at 17 by the sixth adrenal specimen (collected one hour after strychnine). All the bloods were diluted with three volumes Ringer. (Reduced to two-thirds.)

The fourth specimen was assayed at 1:3,000,000, corresponding to an output of 0.0006 mgm. per minute for the cat, or 0.00025 mgm. per kilogram per minute. In this case the reactions of the segment were so definite and so constant in different observations that there could be no doubt that the rate of output was somewhat increased for the fourth specimen. It would be misleading, however, if we did not point out that we cannot in every case, nor indeed in the majority of instances, assay with average segments so closely as to be certain that an output calculated at 0.00025 mgm. per kilogram per minute is really greater than an output calculated at 0.0002 mgm. It is a safe rule in working with such methods of bio-assay to draw no conclusions from apparent small differences in epinephrin output. A difference of 50 per cent on an output of 0.00025 mgm. is usually, a difference of 100 per cent always unmistakable. It is obvious that even with a segment of average sensitiveness and constancy, a good deal must depend upon the absolute concentrations (which themselves depend upon the blood flows) of the various specimens. Where the blood flows of two specimens are the same a comparison of the reactions produced on the segment will sometimes enable the qualitative conclusion to be drawn that one corresponds to a greater epinephrin output than the other, even when the difference may be very small. Still more is this the case when the specimen with the greater concentration has also the greater flow.

The sixth specimen (taken one hour after strychnine when the increase in the reflex excitability had almost disappeared) was shown to be much stronger than 1:2,200,000. As it was so strong it was mixed first with a given proportion of jugular blood and the mixture then diluted with Ringer's solution. In several such observations it was found to be much stronger than 1:1,500,000 and probably somewhat stronger than 1:750,000. It was finally taken at 1:700,000, corresponding to an epinephrin output of 0.0011 mgm. per minute for the cat, or 0.00046 mgm. per kilogram per minute, more than double the initial output. As the concentration was approximately the possible maximum and the blood flow was relatively small, it is probable that the

calculated output is less than would have been obtained at this time with a better flow. With 70 per cent of serum in the blood, the concentration of epinephrin in the serum would be about 1: 500,000, the "possible normal maximum" as estimated on rabbit segments in shed blood.

Strychnine does not, so far as our present experiments show, possess the power of increasing the concentration beyond the normal maximum.

It was not possible, of course, to know whether in this experiment a specimen collected at some time intermediate between the fourth and sixth might not have shown a greater increase in output than the latter.

In the next experiment a somewhat smaller dose of strychnine was employed. It was one of a series of experiments in which the dose was systematically reduced.

Condensed protocol; cat 225; male; weight, 2.05 kgm.

Anesthetized with urethane. Obtained a specimen of indifferent blood from the jugular vein. Made cava pocket. Started artificial respiration. Then collected adrenal blood.

11.30 a.m. First specimen, 1.2 grams in 45 seconds (1.6 grams per minute). Second specimen, 3.7 grams in 180 seconds (1.2 grams per minute).

11.45 a.m. Injected 0.7 mgm. strychnine (in successive doses of 0.3; 0.2; 0.2 mgm). Marked exaggeration of reflexes occurred, but no convulsions.

11.55 a.m. Third specimen, 1.5 grams in 60 seconds (1.5 grams per minute). Fourth specimen, 5.3 grams in 300 seconds (1.06 grams per minute).

12.05 p.m. Closed abdomen with clamps.

12.45 p.m. Fifth specimen, 1.45 grams in 60 seconds (1.45 grams per minute). Sixth specimen, 5.2 grams in 240 seconds (1.3 grams per minute). Reflexes slightly increased.

Obtained another specimen of jugular blood, also a specimen of arterial (abdominal aorta) blood. Combined weight of adrenals 0.292 gram.

Convulsions were not present at any time in this animal, but the reflex excitability was at first markedly increased. The strychnine (0.7 mgm. in all) was injected in successive doses till this occurred. Figure 24 shows that the fourth adrenal blood specimen (collected a few minutes after the last dose of strychnine) caused a much greater inhibition of the intestine segment than the second specimen (collected before strychnine), although the blood flow was only slightly less. The sixth specimen (collected an hour after the first injection of strychnine) had a much greater effect than the fourth, and a very much greater effect

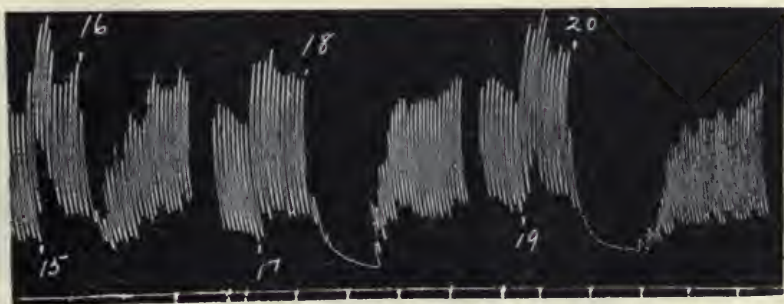


FIG. 24. INTESTINE TRACINGS. BLOODS FROM CAT 225

At 15, 17, and 19 Ringer was replaced by jugular blood, and this at 16 by the second adrenal blood specimen (collected before strychnine); at 18 by the fourth adrenal specimen (collected fifteen minutes after strychnine); at 20 by the sixth adrenal specimen (collected one hour after strychnine). All the bloods were diluted with three volumes Ringer. (Reduced to one-half.)

than the second, although the flow was somewhat greater than for the second specimen. This was confirmed by several other observations. The second specimen was assayed at 1:2,400,000 adrenalin, giving an output of epinephrin of 0.0005 mgm. per minute for the cat, or 0.00025 mgm. per kilogram per minute.

The fourth specimen was assayed at 1:1,600,000, corresponding to an output of 0.0007 mgm. per minute for the cat, or 0.00035 mgm. per kilogram per minute. The assay was such that there was no question that the output was definitely increased at the time of collection of the fourth specimen. The sixth specimen was assayed at 1:1,200,000, corresponding to an

output of 0.001 mgm. per minute for the cat, or 0.0005 mgm. per kilogram per minute, double the original output before strychnine.

In the next experiment, the total dose of strychnine per kilogram of bodyweight was again slightly reduced. The injection was made in successive doses till the reflex excitability became distinctly exaggerated, but there were no convulsions at any stage. Artificial respiration was not required.

Condensed protocol; cat 259; male; weight, 4.1 kgm.

Anesthetized with urethane. Obtained a specimen of indifferent blood from the femoral vein. Made cava pocket. Then collected adrenal blood.

- 11.30 a.m. Blood pressure before collection of adrenal specimen 114 mm. Hg. First specimen, 2.65 grams in 30 seconds (5.3 grams per minute). Second specimen, 9.05 grams in 120 seconds (4.5 grams per minute).
- 11.40 am. Injected 1.25 mgm. strychnine into jugular vein, in three doses (0.5, 0.25, 0.5 mgm.) with short intervals between each dose, until reflexes became distinctly exaggerated.
- 11.47 a.m. Blood pressure before collection of third adrenal specimen 128 mm. Hg. Third specimen, 2.1 grams in 30 seconds (4.2 grams per minute). Fourth specimen, 7.85 grams in 150 seconds (3.14 grams per minute).
- 11.55 a.m. Reflexes markedly exaggerated.
- 12.10 p.m. Reflexes still increased, but less marked than at 11.55. Blood pressure 105 mm. Hg.
- 12.15 p.m. Fifth specimen, 1.8 grams in 30 seconds (3.6 grams per minute). Sixth specimen, 8.1 grams in 180 seconds (2.7 grams per minute).
- 12.45 p.m. Blood pressure 60 mm. Hg.
- 12.50 p.m. Seventh specimen, 1.1 grams in 30 seconds (2.2 grams per minute). Eighth specimen, 5.9 grams in 240 seconds (1.5 grams per minute).

Blood obtained from abdominal aorta. Combined weight of adrenals 0.73 gram.

Figure 25 shows that the inhibitory effect produced on the rabbit intestine segment by the second, fourth, sixth, and eighth adrenal specimens increased progressively in the order named. Since, however, there was also a progressive decrease in the blood flow, no conclusion can be arrived at as to any increase in the epinephrin output from a mere comparison of these tracings. The assay proved that the second specimen (collected before strychnine) had a concentration of 1:4,500,000 epinephrin, corresponding to an output of 0.001 mgm. per minute for the cat, or 0.00025 mgm. per kilogram per minute. The fourth



FIG. 25. INTESTINE TRACINGS. BLOODS FROM CAT 259

At 10, 12, 14, and 16 Ringer was replaced by indifferent (femoral vein) blood, and this at 11 by the second adrenal specimen (collected before strychnine); at 13 by the fourth adrenal specimen (collected eight minutes after strychnine); at 15 by the sixth adrenal specimen (collected thirty-five minutes after strychnine); at 17 by the eighth adrenal specimen (collected seventy minutes after strychnine). All the bloods were diluted with three volumes Ringer. (Reduced to one-half.)

specimen (collected as soon as a distinct effect was produced on the reflex excitability, seven minutes after the first dose of strychnine) had a concentration of 1:3,000,000, giving the same output as the second specimen. At this time there was no demonstrable increase in the output. The sixth specimen (collected thirty-five minutes after the first strychnine injection, when the reflex excitability was still exaggerated, although less than before) was assayed at 1:1,250,000, giving an output of 0.002 mgm. per minute for the cat, or 0.0005 mgm. per kilogram per minute, double the initial output before strychnine. The eighth adrenal specimen (collected seventy minutes after the administration of

strychnine, when the respirations had slowed down considerably and the exaggeration of the reflex excitability had largely passed off) was assayed at 1:700,000 adrenalin, corresponding to an output of 0.002 mgm. per minute for the cat, or 0.0005 mgm. per kilogram per minute, the same as for the sixth specimen and twice the original output before strychnine. The output observed at this time would probably have been greater had the blood flow not diminished so much. For the concentration was at the possible normal maximum. The proportion of serum in the blood was 76.5 per cent, as determined by the electrical method (8) and 73.5 per cent as determined by the haematocrite. The concentration in the serum would, therefore, be 1:530,000.

In this experiment then the increase in the epinephrin output was as great, and probably greater seventy minutes after the administration of strychnine as thirty-five minutes after.

There is good reason to believe that the smaller effect of strychnine in increasing epinephrin output in cats under urethan than in dogs under morphine and ether is largely at any rate due to the difference in the action of the anesthesia on the nervous system in the two cases. The two following experiments illustrate this point.

In a deeply urethanized cat (238), weighing 1.64 kilogram, the intravenous injection of a large dose of strychnine (1 mgm. of the sulphate) caused no convulsions and no increase in the reflex excitability. The output of epinephrin was trebled five minutes after the injection. A further injection of 2 mgm. of strychnine (in two doses) still caused no convulsions and only a slightly increased reflex excitability. The epinephrin output six minutes after the last injection of strychnine was also about three times the original output before the first dose. An adrenal blood specimen collected about fifty minutes after the last strychnine injection still showed about four times the initial output. The fact that strychnine, which under the conditions of this experiment scarcely affects the motor mechanisms of the cord, causes a substantial and sustained augmentation of the epinephrin output has of course a bearing upon the mechanism of the strychnine action upon epinephrin discharge. As regards the question of

the minimum effective dose of strychnine for increasing the epinephrin liberation, the experiment has at least this significance: it shows that it is not necessary that the excitability of the motor reflex paths should be increased. Other experiments confirm this. A more important deduction, however, for our present purpose can be drawn from a comparison of the increase in epinephrin output produced by the very large amount of strychnine administered intravenously in this experiment, with the increase produced by one-twenty-fourth of the amount per kilogram of bodyweight in cat 308. The maximum increase in cat 308 was five times the initial output, a proportional increase greater than in cat 238. The only difference between the two experiments which can account for this is that cat 308 was anesthetized with ether alone and cat 238 with urethane. It seems clear, then, that the effective doses in the urethane experiments are all far greater than would be effective in an etherized or non-anesthetized animal.

Condensed protocol; cat 238; female; weight, 1.64 kgm.

Anesthetized with urethane. Obtained a specimen of indifferent blood from the jugular vein. Made cava pocket. Started artificial respiration (breathing well, spontaneously. Then collected adrenal blood.

- 10.35 a.m. First specimen, 1.3 grams in 45 seconds (1.7 grams per minute). Second specimen, 5.3 grams in 180 seconds (1.7 grams per minute).
- 10.45 a.m. Injected 1.0 mgm. strychnine into jugular vein. Reflexes were not increased. Deeply anesthetized.
- 10.50 a.m. Third specimen, 1.95 grams in 30 seconds (3.9 grams per minute). Fourth specimen, 4.5 grams in 90 seconds (3.0 grams per minute).
- 11.00 a.m. Injected 1 mgm. strychnine into jugular vein.
- 11.04 a.m. Injected 1 mgm. strychnine into jugular vein.
- 11.07 a.m. Slight increase in reflexes—no convulsions.
- 11.10 a.m. Fifth specimen, 1.25 grams in 30 seconds (2.5 grams per minute). Sixth specimen, 4.35 grams in 120 seconds (2.17 grams per minute).
- 11.15 a.m. Closed abdomen with clamps.

11.50 a.m. Seventh specimen, 1.35 grams in 30 seconds (2.7 grams per minute). Eighth specimen, 3.8 grams in 120 seconds (1.9 grams per minute).

Obtained more jugular blood, and a specimen of arterial (abdominal aorta) blood. Combined weight of adrenals 0.288 gram.

Figure 26 shows that the fourth adrenal specimen is decidedly stronger than the second in spite of the much greater blood flow during collection of the fourth. This, of itself proves that the epinephrin output at the time of collection of the fourth specimen was increased. The sixth specimen caused a somewhat greater inhibition of the intestine segment than the fourth. This was more evident in other observations than in observations 4 and 6, figure 26. The eighth specimen caused the greatest inhibition of all. A detailed assay gave for the second specimen a concentration of 1:5,000,000 epinephrin, corresponding to an output of 0.00034 mgm. per minute for the cat, or 0.0002 mgm. per kilogram per minute. The fourth specimen was taken at 1:2,300,000, denoting an output of 0.0013 mgm. per minute for the cat, or 0.0008 mgm. per kilogram per minute. The eighth specimen was assayed at 1:1,750,000, corresponding to an output of 0.00125 mgm. per minute for the cat, or 0.00075 mgm. per kilogram per minute, practically the same as in the fourth specimen.

Condensed protocol; cat 308; male; weight, 3.33 kgm.

Anesthetized with ether. Obtained a specimen of indifferent blood from the jugular vein. Made cava pocket. Then collected adrenal blood.

- 10.35 a.m. Blood pressure 100 mm. Hg.
- 10.36 a.m. First specimen, 1.95 grams in 30 seconds (3.9 grams per minute).
- 10.36½ a.m. Second specimen, 6.7 grams in 120 seconds (3.35 grams per minute).
- 10.44 a.m. Injected 0.25 mgm. strychnine into jugular vein.
- 10.45 a.m. Very slight increase in reflex excitability.
- 10.46 a.m. Slightly greater increase in reflex excitability.

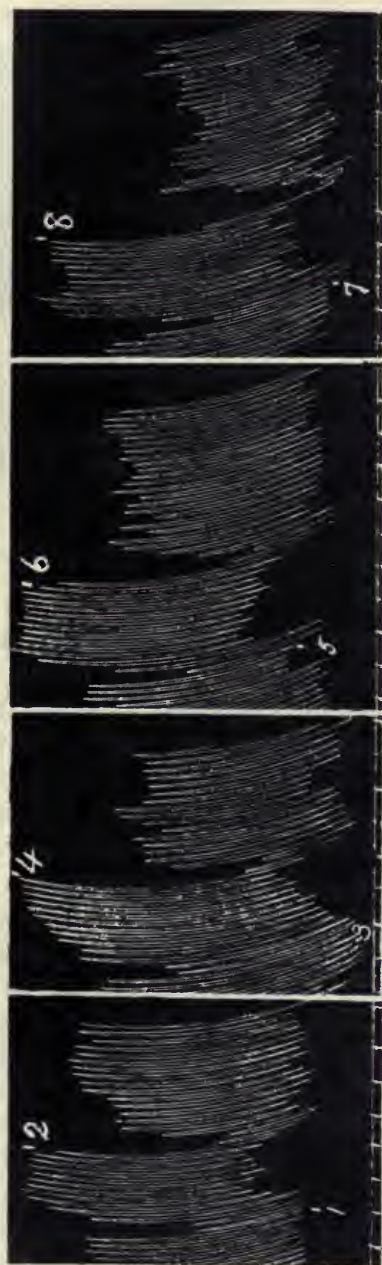


FIG. 26. INTESTINE TRACINGS. BLOODS FROM CAT 238

At 1, 3, 5 and 7 Ringer was replaced by indifferent (jugular) blood, and this at 2 by the second adrenal blood specimen (collected before strychnine); at 4 by the fourth adrenal specimen (collected five minutes after strychnine); at 6 by the sixth adrenal specimen (collected six minutes after two more doses of strychnine); at 8 by the eighth adrenal specimen (collected fifty minutes after the last dose). All the bloods were diluted with three volumes Ringer. (Reduced to one-half.)

- 10.46 a.m. Third specimen, 2.2 grams in 30 seconds (4.4 grams per minute).
- 10.46½ a.m. Fourth specimen, 6.6 grams in 120 seconds (3.3 grams per minute). Blood pressure 86 mm. Hg. Reflexes slightly increased.
- 10.53 a.m. Very little increase in reflex excitability.
- 10.58½ a.m. Fifth specimen, 1.55 grams in 30 seconds (3.1 grams per minute).
- 10.59 a.m. Sixth specimen, 6.9 grams in 150 seconds (2.76 grams per minute). Blood pressure 85 mm. Hg. No increase in reflex excitability.
- 11.31 a.m. Seventh specimen, 1.35 grams in 30 seconds (2.7 grams per minute).
- 11.31½ a.m. Eighth specimen, 6.35 grams in 180 seconds (2.12 grams per minute). Blood pressure 72 mm. Hg. No increase in reflex excitability.

Obtained more venous blood. Combined weight of adrenals 0.473 gram.

The epinephrin assay showed that the second specimen (collected before strychnine) was weaker than the fourth (collected two and one-half minutes after strychnine); the fourth, weaker than the sixth (taken fifteen minutes after the strychnine), and the sixth weaker than the eighth (fig. 27). Since the flows for the second and fourth specimens were the same, the output was already augmented in the fourth specimen and no trace of a preliminary diminution was seen in this experiment. This is instructive, for in another experiment (cat 258) in which the same relatively small dose of strychnine was injected intravenously into a urethanized cat without causing any increase in the reflex excitability, the adrenal blood specimen collected three to four minutes after the strychnine injection had a decidedly smaller concentration of epinephrin (fig. 28, observations 2 and 4, confirmed by other observations) than the specimen obtained before strychnine (1:5,500,000 as compared with 1:4,800,000), although the blood flow was nearly twice as great in the latter. The output of epinephrin was, therefore, distinctly diminished at this time. A given dose of strychnine in a urethanized cat

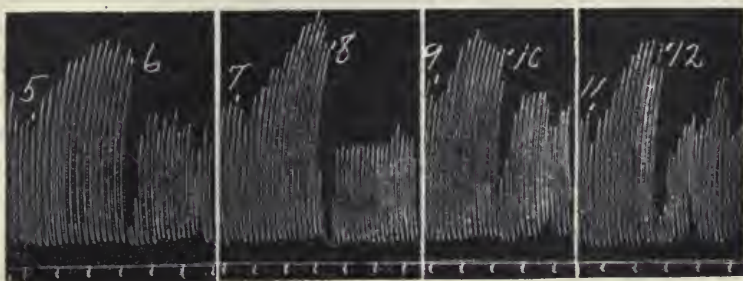


FIG. 27. INTESTINE TRACINGS. BLOODS FROM CAT 308

At 5, 7, 9 and 11 Ringer was replaced by jugular blood, and this at 6 by the sixth adrenal specimen (collected fifteen minutes after injection of strychnine); at 8 by the eighth adrenal specimen (collected fifty minutes after injection of strychnine); at 10 by the second adrenal specimen (collected before injection of strychnine); at 12 by the fourth adrenal specimen (collected two minutes after injection of strychnine). All the bloods were diluted with three volumes Ringer. (Reduced to one-half.)

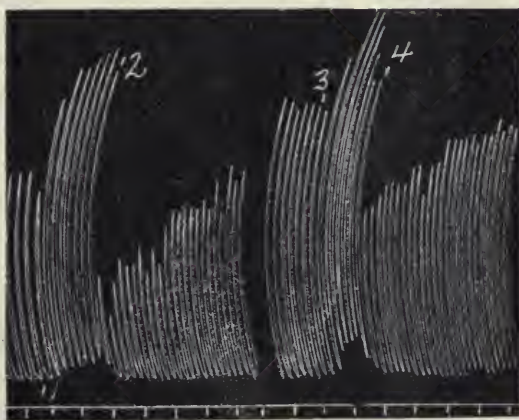


FIG. 28. INTESTINE TRACINGS. BLOODS FROM CAT 258

At 1 and 3 Ringer was replaced by indifferent (femoral vein) blood, and this at 2 by the second adrenal specimen (collected before a small dose of strychnine); at 4 by the fourth adrenal specimen (collected four minutes after the strychnine). The bloods were diluted with three volumes Ringer. (Reduced to one-half.)

would correspond as regards its action on the epinephrin secretion to a smaller dose in an etherized cat.

The second specimen (in cat 308) was found to be decidedly weaker than 1:5,700,000 adrenalin, (confirmed by several observations), and decidedly stronger than 1:8,500,000. It was approximately equal to 1:7,000,000, corresponding to an output of 0.0005 mgm. per minute for the cat, or 0.00015 mgm. per kilogram per minute.

The fourth specimen was much stronger than 1:5,700,000, somewhat stronger than 1:4,300,000. Taking it at 1:4,000,000, the output was calculated at 0.0008 mgm. per minute for the cat, or 0.00025 mgm. per kilogram per minute, nearly twice the initial output. The sixth specimen was assayed at 1:1,200,000, corresponding to an output of 0.0023 mgm. per minute for the cat, or 0.0007 mgm. per kilogram per minute, nearly five times the initial output.

The eighth specimen was assayed at 1:1,000,000, giving 0.0021 mgm. per minute for the cat, or 0.00065 mgm. per kilogram per minute, fully four times the original output. This was nearly fifty minutes after the strychnine injection. The increase in the sixth and eighth specimens was really somewhat greater than stated. For while the second and fourth specimens were assayed in the ordinary routine soon after the bloods were obtained, the assay of the sixth and eighth specimens could not be completed till next day, and although the bloods were kept on ice, some epinephrin must have disappeared. The proportion of serum in the blood was seventy per cent. The serum of the eighth specimen, therefore, contained (twenty-four hours after being drawn) 1:700,000 epinephrin.

GENERAL CONCLUSIONS FROM THE EXPERIMENTS IN WHICH
ADRENAL BLOOD WAS DIRECTLY ASSAYED ON
RABBIT SEGMENTS

In cats, the results were qualitatively the same as in dogs. Quantitatively the maximum increases in the output of epinephrin were in general less in the cat than in the dog. But this was due mainly, if not entirely, to the fact that urethane was the

anesthetic commonly employed for the cats, and with this anesthetic the reflex excitability of the motor mechanisms of the cord was reduced, so that considerably greater doses of strychnine were tolerated without producing convulsions than was the case in the dogs under morphine and ether. Nevertheless, the increases in output in the urethanized cats were quite definite (from two to four times the initial output or even more). In cats, under ether alone, considerably smaller doses of strychnine caused marked effects. As in the case of the dogs, it must be emphasized that doses which caused no convulsions whatever, and only a moderate increase in reflex excitability, were capable of increasing the epinephrin output notably (up to five or six times the original output or more), that the increase persisted a considerable time, that it was often present and sometimes indeed, at its maximum when the heightened motor reflex excitability could no longer be demonstrated.

As regards the length of time during which the increase in the output persists, we have made no attempt to follow it beyond an hour and a half. But at the end of the experiment, it was much more common to find the output still distinctly increased than to find that it had returned to normal, so that it may be concluded with confidence that the effect of a single dose of strychnine is not at all transient.

We have not tried systematically to determine the minimum dose which can produce a definite effect. The necessity of using an anesthetic is, of course, a considerable complication. But since even in anesthetized animals, single doses which reckoned on the bodyweight could be safely used in man have been seen to produce an undoubted and sustained increase in the output, it would seem reasonable to assume that an increased output of epinephrin could be obtained in man by appropriate administration of strychnine, if the production of such an effect should be thought to be indicated for any therapeutic purpose. We express no opinion as to the possible therapeutic applications of the facts described in this paper beyond the suggestion that in such an emergency as cardio-vascular shock, the action of strychnine on the epinephrin output might have some value, of course,

in conjunction with other established methods of treatment, if any methods can be said to be established. It is not without interest that strychnine has been sometimes recommended for treatment of shock without any definite pharmacological basis for the recommendation. Our suggestion is based on indications gradually accumulating in the course of our work, that while the epinephrin output of the adrenals may not under ordinary conditions exert any important action upon the circulation, yet when the circulation is failing, as in shock, and the nervous regulation which plays normally the predominant rôle, has become totally inadequate, the *relative* importance of the epinephrin factor may be enhanced. At this moment a marked and sustained increase in the epinephrin output might intervene, we will not say as an obviously decisive factor, but as a sustaining influence, especially upon the heart, which might help to turn the balance in the right direction.

We have not infrequently had the opportunity to observe that in these circumstances it was especially easy to demonstrate that the normally secreted epinephrin was really not without influence upon the heart and that strychnine seemed to produce an augmentation of this influence.

We do not subscribe to the view expressed by a number of investigators and most recently by Gley (3) that the epinephrin normally liberated from the adrenals cannot be shown to exert any action whatever. It is quite certain that it is not indispensable (19). Nor is there sufficient reason for the view that an augmented epinephrin liberation is a factor in eliciting any of the bodily changes associated with emotional disturbances, or in the increased mobilization of sugar in certain experimental hyperglycemias. But it is going too far, we believe, in the other direction to assert that the naturally secreted epinephrin produces no detectable effect at all. The effects which it does produce are not gross effects which it is easy to detect. Nor is it possible in the present state of our knowledge to assign to any of them a rôle of definite physiological significance. But we have seen, and to some extent studied, two or perhaps three reactions which demonstrate that the amount of epinephrin spontaneously

given off by the adrenals, under our experimental conditions, does exert an appreciable action upon certain structures.

One of these reactions, on the pupil after removal of the superior cervical ganglion, has already been described (7).

The second is the effect upon the heart-beat, which has just been alluded to and which will come more appropriately in another paper. A third reaction, on which further study is necessary, concerns the relation of the normally secreted epinephrin to the rate of the heart. We have obtained some evidence that such changes in the amount and concentration of the naturally secreted epinephrin passing through the coronary vessels as can be produced by altering the distribution of the blood through stimulation of vasomotor nerves and in other ways, can affect the rate of the heart, without any change having been caused in the rate of output of epinephrin (17). Further evidence of a relation of the normal epinephrin output to the heart rate seems to be afforded by a comparison of the effects produced in cats on the rate by section of the accelerantes (excision of the stellate ganglia) after previous section of the vagi, when the adrenal epinephrin is normally entering the circulation and in the absence of epinephrin. It seemed easier to demonstrate a decided slowing of the heart after section of the accelerantes in the absence of an epinephrin secretion. The suggestion is that when the nervous influence is eliminated the stimulation produced by the epinephrin still suffices to maintain the heart rate at a higher level than in the absence of epinephrin. Further work, however, is necessary in regard to this last test.

It is of course possible that if the adrenal medulla or cortex forms and gives off to the blood other and perhaps more important substances than epinephrin, the output of these may also be influenced by the action of strychnine upon the nervous mechanism. Our experiments naturally throw no light upon this, but the possibility should be borne in mind.

Table 1 summarizes the results obtained with segment assays which are not complicated by the repetition of the initial dose of strychnine. In addition to adrenal specimens collected after strychnine in experiments where only one dose was given, speci-

TABLE 1

NUMBER OF ANIMAL	WEIGHT	STRYCHNINE IN- JECTED	STRYCHNINE PER KILOGRAM	BEFORE INJECTION OF STRYCHNINE						AFTER INJECTION OF STRYCHNINE						Blood col- lected after
				Adrenal blood collected	Length of col- lection	Flow per min- ute	Concentration of epineph- rin	Output per minute	Output per kilogram per minute	Adrenal blood collected	Length of col- lection	Flow per min- ute	Concentration of epineph- rin	Output per minute	Output per kilogram per minute	
	kgm.	mgm.	mgm.	grams	seconds	grams		mgm.	mgm.	grams	seconds	grams		mgm.	mgm.	minutes
245*	9.5	4.0	0.421	10.0	60	10.0	1: 3,750,000	0.0027	0.00028	12.15	60	12.15	1:2,500,000	0.0048	0.0005	5
246*	7.5	2.0	0.266	7.7	30	15.4	1:13,000,000	0.0012	0.00016	8.3	30	16.6	1:1,500,000	0.011	0.0015	2
248*	4.6	0.5	0.11	9.7	60	9.7	1:13,000,000	0.0008	0.00017	8.9	60	8.9	1:1,250,000	0.007	0.0015	2
263*	4.4	0.25	0.056	6.15	60	6.15	1: 3,800,000	0.0016	0.00036	5.7 5.75 7.4	60 60 120	5.7 5.75 3.7	1:4,300,000 1:2,500,000 1:1,100,000	0.0013 0.002 0.0034	0.0003 0.00045 0.00075	2 20 85
306* †	5.05	2.0	0.4	8.2	60	8.2	1: 8,500,000	0.001	0.0002	16.1 9.55 7.55	60 60 60	16.1 9.55 7.55	1:1,600,000 1:1,200,000 1:4,700,000	0.01 0.008 0.0016	0.002 0.0016 0.0003	10 30 50
309* †	8.1	0.75	0.092	15.9	60	15.9	1: 6,100,000	0.0026	0.00032	11.1 10.3	60 60	11.1 10.3	1:2,300,000 1:2,000,000	0.0048 0.0051	0.0006 0.00063	20 50
225	2.05	0.7	0.35	3.7	180	1.2	1: 2,400,000	0.0005	0.00025	5.3 5.2	300 240	1.06 1.3	1:1,600,000 1:1,200,000	0.0007 0.001	0.00035 0.0005	15 60

228	2.5	1.0	0.4	5.2	90	3.5	1: 5,800,000	0.0006	0.00024	{	6.7	120	3.3	1:3,300,000	0.001	0.0004	2
											6.75	120	3.4	1:1,650,000	0.002	0.0008	45
238	1.64	1.0	0.61	5.3	180	1.7	1: 5,000,000	0.00034	0.0002	4.5	90	3.0	1:3,000,000	0.001	0.0006	5	
239	2.41	1.0	0.41	4.6	120	2.3	1: 5,000,000	0.00046	0.0002	{	3.5	120	1.75	1:3,000,000	0.0006	0.00025	3
											3.05	240	0.76	1: 700,000	0.0011	0.00046	60
308	3.33	0.25	0.075	6.7	120	3.35	1: 7,000,000	0.0005	0.00015	{	6.6	120	3.3	1:4,000,000	0.0008	0.00025	2
											6.9	150	2.76	1:1,200,000	0.0023	0.0007	15
											6.35	180	2.12	1:1,000,000	0.0021	0.00065	50

* First 6 are dogs, the others are cats.

† Strychnine administered subcutaneously; all others intravenously.

Cat 308 was anesthetized with ether, the other cats with urethane, the dogs with morphine and ether.

mens collected before repetition of the dose in experiments where it was repeated are included.

Confirmatory evidence that strychnine increases the rate of epinephrin liberation was sought by comparing the effects of given doses of strychnine on the eye, after previous removal of the superior cervical ganglion, in otherwise normal cats and in cats whose epinephrin output had been interfered with by excision of one adrenal and section of the nerves of the other. It has been already pointed out that indirect observations of this kind are more difficult to interpret and, therefore, less trustworthy than assays of the drawn adrenal blood by means of rabbit segments, or than auto-assays of the blood collected in a cava pocket by means of the blood pressure reaction which follows release of the blood into the circulation. We should never think of concluding from the eye observations alone that the output of epinephrin was certainly increased by strychnine, still less of attempting to estimate the amount of the increase. But as confirming the results of the direct methods these observations have a distinct value, for they showed that the (denervated) eye reactions (especially the dilatation of the pupil) induced by strychnine were decidedly greater and lasted much longer in the normal animals than in animals which had been subjected to the adrenal operation.

It must be distinctly pointed out that in the operated cats, the pupil of the denervated eye always remained larger than the pupil of the normal eye, for a longer or shorter time after a strychnine convulsion just as in the normal cats. The difference is not a qualitative, but a quantitative one. It is a pretty general rule that any influence which causes dilatation of the normal pupil (except of course excitation of sympathetic pupillo-dilator fibers) causes a still greater or more permanent dilatation of the pupil on the side from which the superior cervical ganglion has been removed, and the suppression of the epinephrin output does not alter this rule. We have had occasion to observe this in all sorts of experiments. Erroneous conclusions have been drawn by various writers as to the influence of certain factors upon the epinephrin discharge because this has not been taken

into account. Let it be repeated, it is only because of the apparently marked quantitative difference in the eye reactions produced by strychnine in the absence of the epinephrin secretion that we venture to draw any deduction from these observations as to the effect of the drug upon the epinephrin output, and then only by way of corroborating results obtained by more certain methods.

Experiments were made on four cats in which the adrenal operation had been performed ten to twenty-one days previously, and which had completely recovered. The left superior cervical ganglion had been removed at the same time. Three normal cats, from which the left superior cervical ganglion had been removed ten to fifteen days before the strychnine experiments, were used as controls. On two of these normal cats the experiment was repeated on another day, with the same result. All the animals were females. The strychnine was injected subcutaneously. No anesthetic was used, except at the close of the observations on the adrenal cats when the residual epinephrin output was to be determined. The best idea of the difference caused by the adrenal operation can be given by quoting the condensed protocols of a typical experiment from each group.

Condensed protocol; cat 277; female, weight, 1.75 kgm.

Twenty-one days before the experiment the right adrenal was excised, the left adrenal denervated and the left superior cervical ganglion excised. The right adrenal weighed 0.28 gram and contained 0.25 mgm. epinephrin. Left pupil contracted and nictitating membrane forward.

10.10 a.m. 0.5 mgm. strychnine injected hypodermically.

10.20 a.m. Cat spastic; reflexes markedly exaggerated, excitation brings on spasticity but no convulsions; no change in pupils or nictitating membrane.

11.08 a.m. 0.25 mgm. strychnine injected hypodermically.

11.12 a.m. Condition same as at 10.20, but more marked; on excitation clonic spasms occurred and during these attacks the left nictitating membrane retracted, the pupils became about equal and maintained equality whether dilatation or contraction was occurring.

- 11.35 a.m. Excited reflexes in cat (tapping) until a tonic convulsion occurred; this at first caused no change from the observations mentioned above, but when asphyxia came on, both pupils dilated to maximum; artificial respiration was at once begun and for 5 to 6 minutes no spontaneous respirations occurred, the heart was very slow and the pupils maximal; as spontaneous respiration came on, the pupils gradually came down, the left remaining slightly wider than the right; the heart rate increased; within 5 to 10 minutes the pupils were equal and finally the left became smaller than the right. On excitation the left pupil became slightly wider than the right, both dilating, but at once came down to its previous condition.
- 12.00 m. Urethane administered; obtained (jugular) indifferent blood; made cava pocket and collected two specimens of adrenal vein blood (blood flow 0.22 gram per minute). The left adrenal weighed 0.293 gram and contained 0.22 mgm. epinephrin. The second adrenal blood specimen assayed at 1:4,000,000, corresponding to an output of 0.00003 mgm. per kilogram per minute, or about one-eighth of the normal average output.

The output of epinephrin determined in adrenal blood collected at the end of the eye observations was greater in this cat than is usual after the adrenal operation. It is not known whether the strychnine was still causing a relatively increased output through fibres which had escaped section at the operation. If so, this would account for the magnitude of the residual liberation. In any case, however, if epinephrin is an important factor in producing and maintaining the great dilatation of the pupil of the denervated eye in the normal animals under the influence of strychnine, the suppression of seven-eighths of the output must be sufficient to cause a great difference in the cat which had undergone the adrenal operation.

Condensed protocol; cat 270; female; weight, 2.5 kgm.

Left superior cervical ganglion excised ten days previously. Left pupil contracted and nictitating membrane forward.

- 2.40 p.m. 0.5 mgm. strychnine injected hypodermically.
2.45 p.m. 0.25 mgm. strychnine injected hypodermically.
2.48 p.m. Tonic convulsion lasting about one-half minute; no artificial respiration was needed. In about 16 seconds from the onset of the convulsion the left pupil became maximal and the nictitating membrane retracted; the right pupil dilated also but not nearly so widely as the left and soon came down again while the left remained maximal for a long while.
4.00 p.m. Cat still spastic; reflexes exaggerated; left pupil much larger than right.
4.30 p.m. Cat quiet, but reflexes are still increased; left pupil is slightly larger than right.
5.00 p.m. Pupils are about equal; when excited, both pupils dilate, but the left becomes somewhat wider than the right, soon coming down to equality again.

EXPERIMENTS ON THE INFLUENCE OF STRYCHNINE ON THE EPINEPHRIN STORE OF THE ADRENALS

We have several times pointed out that changes in the epinephrin store are no certain index of changes in the rate of output of epinephrin. This is very well illustrated by four experiments on cats, in which the effect of the strychnine on the store was investigated. The left adrenal was denervated nine to fourteen days prior to the experiment. In three of the cats the left superior cervical ganglion was also excised, so that the eye reactions could be observed under strychnine. The cats were kept thoroughly under the influence of strychnine for several hours and then killed suddenly by a blow. The adrenals were immediately removed and the amount of epinephrin in each estimated by the colorimetric method of Folin, Cannon and Denis. The doses of strychnine used were much larger than would have been necessary even in anesthetized animals to produce a marked and sustained increase in the epinephrin output and strong convulsions were always induced, artificial respiration by means of the apparatus described by us for use in man (18) being given when necessary to prevent asphyxia during the spasms. As much strychnine was given as was compatible with survival of the

animals for the requisite time. Indeed, a fifth cat died forty minutes after the first dose. Yet in none of the animals could any definite difference, beyond the range of the ordinary variations, be made out in the store of the denervated adrenal as compared with its fellow. In the one experiment made by Elliott (5) the same result was obtained. The formation of epinephrin must, therefore, have been increased in approximately the same measure as its liberation. This is precisely what is seen when the output is increased by stimulation of the splanchnic nerves. It is what might be expected of a secretory nervous mechanism, regulating the output of a substance which is given off constantly to the blood, and we have good evidence that it is through the nervous mechanism and not through any direct effect upon the glands that strychnine increases the epinephrin output. It has been stated in another paper (19) that in an animal in which the epinephrin output has been much diminished or abolished by removal of one adrenal and denervation of the other, strychnine does not cause epinephrin to be liberated although the store in the adrenal is of normal magnitude. That in the absence of the innervation the adrenal medulla is capable of accumulating epinephrin till the normal store is reached, has been clearly demonstrated, first of all by Elliott (5). This, however, is not in any way inconsistent with the speeding up through the nervous mechanism of the process of formation and accumulation when the output is increased.

This speeding up could take place through the intervention of special nerves. Or without this the mere liberation of a small part of the store through the secretory nerves may stimulate the medullary cells to increased accumulation till the normal load has been reached, when in the absence of liberation the accumulation would automatically cease. This would explain why, in the case of the denervated gland, although a depleted store is soon filled up, there is no demonstrable overflow of epinephrin into the blood.

As the experiments on the effect of strychnine upon the epinephrin store in the cats with one superior cervical ganglion excised afforded the opportunity of observing the effect of strychnine

nine upon the denervated eye reactions in animals whose epinephrin output could only be increased half as much as in normal cats, one typical protocol is quoted.

Condensed protocol; cat 261; male; weight, 1.5 kgm.

Left adrenal denervated 12 days before the experiment. Left superior cervical ganglion excised 9 days before the experiment. Left pupil contracted and nictitating membrane forward.

10.20 am. 0.5 mgm. strychnine injected hypodermically.

10.25 a.m. Marked increase in reflex excitability.

10.30 a.m. Tonic convulsion; both pupils dilated widely, but left more than right; after the convulsion the right pupil came down, but the left remained maximal.

10.35 a.m. Left pupil wider than right and nictitating membrane forward; right nictitating retracted.

10.40 a.m. Pupils about equal; cat still spastic; excitement does not cause the left pupil to become larger than the right.

10.45 a.m. Left pupil smaller than right; remains smaller than right when both dilate on excitation of animal.

11.50 a.m. 0.25 mgm. strychnine injected hypodermically.

11.55 a.m. Tonic convulsion; same phenomena as described under 10.30 observation occurred.

1.40 p.m. Effect of strychnine apparently worn off; 0.25 mgm. strychnine injected hypodermically.

1.45 p.m. Tonic convulsion with same phenomena as described above.

3.30 p.m. 0.25 mgm. strychnine injected hypodermically.

3.35 p.m. Tonic, then clonic spasm; same phenomena observed.

4.00 p.m. Still quite spastic; killed by a sudden blow on head.

Left adrenal weighed 0.18 gram and contained 0.19 mgm. epinephrin. Right adrenal weighed 0.16 gram and contained 0.15 mgm. epinephrin.

In these cats the effects on the denervated eye seemed to be greater and more lasting than in the cats with one adrenal removed and the other denervated, but smaller and more transient than in the normal cats.

The results of the experiments on the effect of strychnine on the epinephrin store are given in table 2.

TABLE 2

NUMBER OF ANIMAL	WEIGHT	WEIGHT OF ADRENALS		EPINEPHRIN		TIME AFTER OPERA- TION	STRYCHNINE	
		Left	Right	Left	Right		Total dose	Duration of action.
	<i>kgm.</i>	<i>grams</i>	<i>grams</i>	<i>mgm.</i>	<i>mgm.</i>	<i>days</i>	<i>mgm.</i>	<i>hours</i>
251	2.16	0.14	0.15	0.16	0.16	12	1	5
253	2.87	0.26	0.248	0.22	0.18	14	1.25	5
261	1.5	0.18	0.16	0.19	0.15	12	1.25	6
271	2.1	0.147	0.15	0.17	0.17	9	0.75	$\frac{1}{2}$
272	1.32	0.108	0.12	0.12	0.12	9	0.5	4

SUMMARY

1. The paper begins with a discussion of essential points in the technique of measuring the epinephrin output. It is pointed out that in general it is no more possible to demonstrate (or measure) alterations in the rate of epinephrin output by observations which only take account of changes in the concentration of epinephrin in the blood coming from the adrenals, while ignoring concomitant changes in the rate of the blood flow, than it would be to demonstrate (or measure) alterations in the rate of carbon dioxide production in an organ by observations which only took account of the number of volumes of carbon dioxide in 100 cc. of blood, but paid no attention to the number of cubic centimeters of blood passing through the organ in a given time. In this connection it is again shown that statements in the literature as to the influence of various conditions in notably augmenting the rate of epinephrin output are vitiated by neglect of this factor.

2. Strychnine causes a marked increase in the output of epinephrin from the adrenals (in the dog and cat). Although it is only by chance that a sample of adrenal blood corresponding to the maximum increase can be collected, outputs ten times the original output have been observed.

3. The increase is not transient but persists for a considerable time. No attempt was made to continue the experiments until it had completely subsided, as it was not thought that any useful purpose could be served by keeping the animals, after the necessary operation, several hours under the anesthetic. The last

adrenal sample was usually taken an hour to an hour and a half after the strychnine injection and it was the rule to find that at this time the epinephrin output was still notably augmented. Indeed, with the smaller doses the effect may go on increasing during the whole experiment and the last specimen may correspond to a rate of output as great as or greater than that of any previous specimen. Abundant evidence has been produced in other papers that animals under similar experimental conditions, but without strychnine, do not show an increased epinephrin output.

4. No attempt was made to fix a minimum effective dose but it was clearly shown that doses of strychnine well within the therapeutic range, and which caused little or no exaggeration of reflex excitability are capable of producing a considerable augmentation in the rate of output. In this connection it must be remembered that the animals were necessarily well anesthetized, and it is to be supposed that still smaller doses would suffice in non-anaesthetised animals.

5. Indications were obtained in some experiments that the stage of prolonged augmentation of the rate of output, which constitutes the principal action of the drug, may be preceded by a transient diminution. This phenomenon was best seen with the smaller doses and with subcutaneous administration of the drug, presumably because with the larger doses and with intravenous injection the augmentation of the output comes on so rapidly as to mask any preliminary decrease.

6. The augmentation of the output caused by strychnine is associated with a more or less marked increase in the epinephrin concentration, even when at the same time the rate of blood flow through the adrenals has been increased, a phenomenon not seen in the absence of the drug. But no evidence has been found that under the influence of strychnine the possible normal maximum concentration in the plasma (something like 1:500,000 as assayed by rabbit segments in adrenal blood from non-strychninised animals) can be increased.

7. The above conclusions are all based on assays of adrenal blood with rabbit intestine and uterus segments. But corroboration

rative evidence of the augmenting influence of strychnine was obtained by studying the effects produced on the blood pressure by adrenal blood, collected in a cava pocket for a given time before and after strychnine, when the blood was allowed to pass from the pocket into the circulation, and in other ways.

8. In spite of the greatly increased output of epinephrin caused by strychnine, there was no evidence that the epinephrin store of the adrenals is distinctly diminished even by the prolonged action of the drug in large and repeated doses. The accumulation of epinephrin in the glands is therefore increased as well as its liberation. This is what happens during stimulation of the splanchnic except when intermittent stimulation is continued for very long periods. It corroborates other evidence that the strychnine effect is produced by an intensification of the secretory process through the nervous mechanism which normally governs it. There is no direct action on the glands.

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THE ACTION OF DRUGS ON THE OUTPUT OF EPINEPHRIN FROM THE ADRENALS

II. CONCENTRATED SALT SOLUTIONS (SODIUM CARBONATE) INJECTED INTO THE CIRCULATION

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Our attention was accidentally drawn to the action upon the epinephrin output of small quantities of concentrated salt solutions introduced directly into the circulation of cats. We were using sodium carbonate solution (half to three-quarters saturated) in the tube connecting the carotid with the mercurial manometer. We were determining the epinephrin output under conditions in which a considerable fall of blood pressure was liable to occur some time after the collection of the adrenal blood specimens on which the normal or initial epinephrin output was estimated. In occasional experiments we were puzzled to find that one or more of the subsequent adrenal specimens had epinephrin concentrations so much out of line with the usual rule, that the concentration is inversely proportional to the blood flow, that it was necessary to assume that the rate of epinephrin liberation had undergone an abrupt and decided change. This had not occurred in long series of experiments made under other conditions in which blood pressure tracings were not necessary. On looking into the matter it was observed that when anomalous behavior of the epinephrin output occurred the fall of blood pressure was speedily followed by an abrupt rise associated with evidence of excitation of the central motor mechanisms (increased reflex excitability and convulsions and changes in the

respiration). It is known that the intravenous injection of concentrated salt solutions leads to those symptoms (1), (2), (3). It seemed fairly clear, then, that small quantities of the carbonate solution passing back from the manometer connection into the artery must have been responsible not only for the motor and vasomotor excitation, but also for the changes in the epinephrin output, presumably through an action on the central nervous mechanism which governs it.

For instance, in one experiment (cat 208) in which it had been intended to follow the epinephrin output in vascular shock, on taking the clip off the carotid in order to begin a blood pressure tracing before the first adrenal specimen was collected the blood pressure was lower than expected and a little of the carbonate solution entered the artery. The blood pressure rose rapidly from 100 to 188 mm. of mercury, with symptoms of excitation of the cord and bulb. The adrenal specimen now collected (the second) was shown, by an assay on rabbit segments, to have a concentration of epinephrin greater than 1:3,300,000, less than 1:1,700,000, and approximately equal to 1:2,500,000, corresponding to an output of 0.0016 mgm. per minute for the cat, or 0.00065 mgm. per kilogram per minute. This is two to three times the normal average, and although no specimen had been obtained before the entrance of carbonate with which to compare it, there is no doubt that the output at the time of collection of the second specimen had been decidedly increased. The fourth adrenal specimen (collected about an hour thereafter, when the blood pressure was only 40 mm. of mercury), had a concentration of 1:2,500,000, the same as that of the second specimen, although the blood flow was not more than one-eighth of that during collection of the second specimen, and the output per kilogram per minute was only 0.00008 mgm. .

As a specimen of adrenal blood was not obtained before the entrance of the carbonate, it is impossible to know whether at the time of collection of the fourth specimen the output had regained the initial amount or was still below it.

Condensed protocol. Cat 208; male; weight, 2.4 kgm.

Anesthetized with urethane. Obtained specimen of indifferent blood from jugular. Made cava pocket.

10.22 a.m. Blood pressure 100 mm. Hg. On opening the carotid clip to take the blood pressure some saturated carbonate solution entered the artery and at once the blood pressure rose to 188 mm. Artificial respiration was started and kept up throughout the experiment.

10.26 a.m. Blood pressure 160 mm. Hg. Collected adrenal blood. First specimen, 3.6 grams in 45 seconds (4.8 grams per minute). Second specimen, 10.1 grams in 150 seconds (4.04 grams per minute).

11.13 a.m. Blood pressure 50 mm. Hg. Collected adrenal blood from pocket. Third specimen, 0.9 gram in 60 seconds (0.9 gram per minute). Fourth specimen, 4.1 grams in 480 seconds (0.5 gram per minute).

Obtained more indifferent (venous) blood. Combined weight of adrenals 0.467 gram.

On putting the matter to an experimental test it proved easy to demonstrate that the injection of small quantities of a strong solution of the salt into the blood stream caused a distinct increase in the epinephrin output, simultaneous with the rise of blood pressure and the convulsions. The cardio-inhibitory center was excited and sometimes the vomiting center. The apnoea which is known to follow the intravenous administration of the carbonate was guarded against by artificial respiration, so that asphyxia played no part in the phenomena. As was to be expected, owing to the greater concentration in which the salt arrives at the nervous centres, the greatest effects were elicited by intraarterial injection. A typical experiment (cat 211) follows.

Condensed protocol. Cat 211; female; weight, 2.8 kgm.

Anesthetized with urethane; cannula in carotid for blood pressure.

11.10 a.m. Cava pocket made with cannula pushed into it through renal vein (left) for collection of adrenal blood. The abdominal aorta was not tied off.

- 11.35 a.m. Collected first adrenal specimen, 1.75 grams in 60 seconds (1.75 grams per minute). Second adrenal specimen, 3.5 grams in 240 seconds (0.9 gram per minute).
- 11.45 a.m. Obtained indifferent (jugular) blood.
- 12.04 p.m. Started artificial respiration and kept it up throughout experiment. Inserted cannula into lower end of pocket; clipped off abdominal aorta. Blood pressure before injection of carbonate 90 mm. Hg. Injected *via* carotid artery 5 to 6 cc. half-saturated carbonate solution. Blood pressure just after injection of carbonate 145 mm. Hg. The blood pressure rose to 228 mm. and a third specimen of adrenal blood was at once collected while blood pressure was 200 mm., and a fourth specimen while blood pressure was 180 mm. Hg. Third adrenal specimen, 6.4 grams in 90 seconds (4.2 grams per minute). Fourth adrenal specimen, 7.55 grams in 210 seconds (2.3 grams per minute).

Indifferent blood was now obtained from the abdominal aorta. On dilution with Ringer the arterial blood clotted readily, but only with difficulty before dilution. Combined weight of adrenals 0.580 gram.

The assay showed that the second adrenal specimen (collected before injection of carbonate) was stronger than 1:3,600,000, somewhat weaker than 1:1,800,000. It was finally taken at 1:2,000,000 epinephrin, equivalent to an output of 0.00045 mgm. per minute for the cat, or 0.00016 mgm. per kilogram of body-weight per minute. The fourth adrenal specimen (collected about two minutes after injection of the carbonate) was found to be stronger than 1:1,400,000, and weaker than 1:900,000 adrenalin. It was assayed at 1:1,200,000 corresponding to an output of 0.002 mgm. per minute for the cat, or 0.0007 mgm. per kilogram per minute. At this time the output was accordingly increased three to four times. The third adrenal specimen, collected just before the fourth, was somewhat stronger than the latter, although the blood flow during its collection was almost twice as great. The output of epinephrin at this time must accordingly have been at least eight times the original output before sodium carbonate was administered. As in the case of the other substances investigated (strychnine, nicotine, etc.),

indifferent blood, obtained after injection of carbonate, was always used in the assay of the adrenal blood specimens taken after carbonate administration, as well as the indifferent (venous) blood drawn at the beginning of the experiments. The carbonate indifferent blood was always obtained from a vein, except when the pressure had sunk too low at the end of an experiment, when it was taken from an artery.

In the next experiment (cat 214) the carbonate was injected not directly into an artery, but into the jugular vein. At the same time a smaller dose was employed, namely 1 cc. of the saturated solution.

Condensed protocol. Cat 214; male, weight, 2.35 kgm.

Anesthetized with urethane. Obtained indifferent blood from jugular. Made cava pocket.

10.46 a.m. Artificial respiration started and kept up throughout experiment.

10.48 a.m. Collected adrenal blood. First specimen, 2.4 grams in 60 seconds (2.4 grams per minute). Second specimen, 7.0 grams in 180 seconds (2.3 grams per minute). Blood pressure 84 mm. Hg.

10.58 a.m. Injected 1.0 cc. saturated solution sodium carbonate into external jugular vein. Blood pressure 85 mm. Hg. Gasping and vomiting movements.

11.00 a.m. Third adrenal specimen, 3.45 grams in 45 seconds (5.0 grams per minute). Fourth adrenal specimen, 6.65 grams in 120 seconds (3.3 grams per minute). Blood pressure during collection of third specimen 158 mm. Hg. Blood pressure during collection of fourth specimen 136 mm. Hg.

11.10 a.m. Obtained more jugular blood.

11.57 a.m. Fifth adrenal specimen, 1.2 grams in 60 seconds (1.2 grams per minute). Sixth adrenal specimen, 4.2 grams in 240 seconds (1.05 grams per minute). Blood pressure 56 mm. Hg.

Indifferent blood obtained from abdominal aorta. Combined weight of adrenals 0.348 gram.

Naturally the rise of blood pressure did not begin so soon as with intraarterial injection (fig. 1). nor was it so abrupt. After a moderate initial rise (to a maximum of 124 mm. of mercury) a marked inhibition of the heart ensued, accompanied by a brief, but great fall of pressure (to 26 mm.) This was at once succeeded by a great increase of pressure (to a maximum of 188 mm. of mercury). Asphyxia was completely excluded by artificial respiration, begun before injection of the carbonate. The blood pressure maintained itself at a high level for about two minutes. The three or four considerable and sudden depressions of the curve at this time were concomitant with gasping and vomiting movements. The beginning of collection of the third adrenal blood specimen was at 3, and forty-five seconds later the collection of the fourth specimen was begun (three minutes after injection of the carbonate), the blood pressure being still 135 mm. of mercury.

The assay showed that the second adrenal specimen (collected before injection of the carbonate) had a concentration greater than 1:3,500,000, less than 1:1,700,000. It was finally assayed at 1:2,500,000 epinephrin, equivalent to an output of 0.0009 mgm. per minute for the cat, or 0.0004 mgm. per kilogram per minute. The fourth adrenal specimen (collected three minutes after administration of the carbonate, or two and a half minutes if allowance is made for the dead space in the cannula and cava) was found to be stronger than the second specimen, in spite of the greater blood flow. It was weaker than 1:1,300,000 adrenalin, much stronger than 1:3,500,000, approximately equal to 1:1,700,000, corresponding to an output of 0.002 mgm. per minute for the cat, or 0.00085 mgm. per kilogram per minute, about twice the original output before the carbonate was injected. The sixth specimen (collected an hour after injection of carbonate when the blood pressure was only 56 mm. of mercury) was shown to be somewhat stronger than 1:850,000, and weaker than 1:700,000 adrenalin. It was taken at 1:800,000, corresponding to an output of 0.0012 mgm. per minute for the cat, or 0.0005 mgm. per kilogram per minute, about the same as for the second specimen. With 71 per cent of serum in the

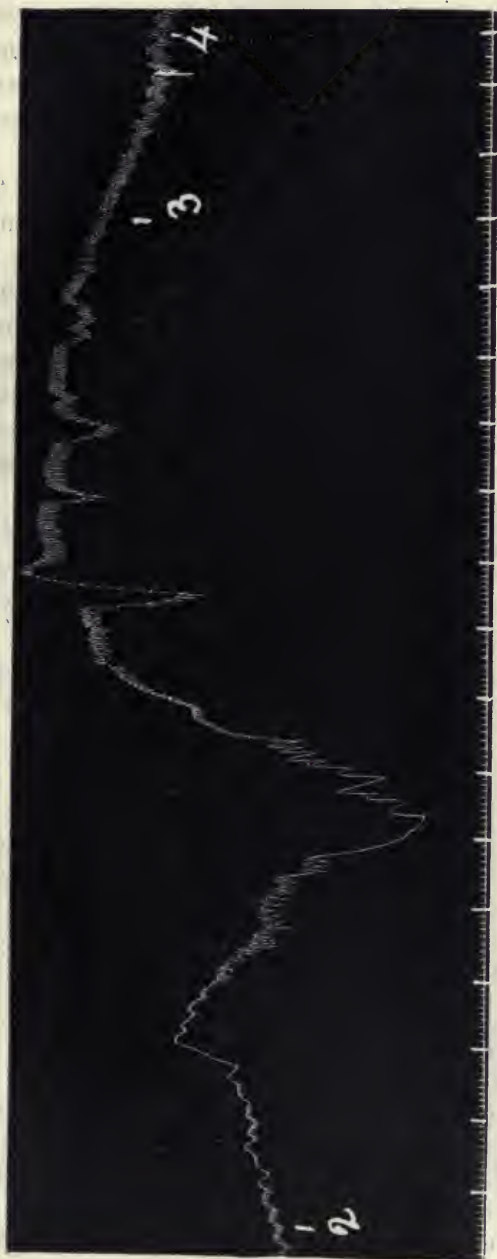


FIG. 1. BLOOD PRESSURE TRACINGS

Cat 214. 2, beginning of carbonate injection; 3, beginning of collection of third adrenal specimen; 4, beginning of collection of fourth adrenal specimen. Time trace, ten second intervals. Line of zero pressure coincides with time trace. (Reduced to two-thirds.)

blood, the concentration of the serum in the sixth specimen would be 1: 500,000, the "possible normal maximum concentration." It has not been shown whether this maximum can be transcended during the increased liberation of epinephrin caused by carbonate injection.

Figure 2 shows that the fourth specimen was stronger than the second, despite the considerably greater blood flow when the fourth was collected. This of itself would prove that the output of epinephrin was increased at this time. The sixth specimen had a much greater concentration than the fourth (fig. 2, observation 6), but as the flow was only one-third as great, nothing can be deduced from the concentration alone as to the output. As already stated, it was shown by the assay that it was not much more than half as great when the sixth specimen was being taken as when the fourth was being collected.

Although there could be little doubt that the effect of the carbonate on the epinephrin output was due to the stimulating action of a strong salt solution on the nervous mechanism which governs the secretion, the possibility could not be ignored that the production of a marked alkalosis might itself more directly affect the rate at which epinephrin was liberated from the adrenals.

In the next experiment (cat 212) a quantity of carbonate a little greater, reckoned on the bodyweight, than that given to cat 214, was injected into the jugular vein, but instead of 1 cc. of the saturated solution, 10 cc. of a 5 per cent solution was administered.

Condensed protocol. Cat 212; female; weight, 2.2 kgm.

Anesthetized with urethane. Obtained indifferent blood from jugular. Made cava pocket.

12.05 p.m. Collected adrenal blood. First specimen, 2.3 grams in 60 seconds (2.3 grams per minute). Second specimen, 4.3 grams in 180 seconds (1.43 grams per minute). Started artificial respiration and kept it up throughout experiment.

12.18 p.m. Injected 10 cc. of 5 per cent sodium carbonate solution into jugular vein; gasping respirations.

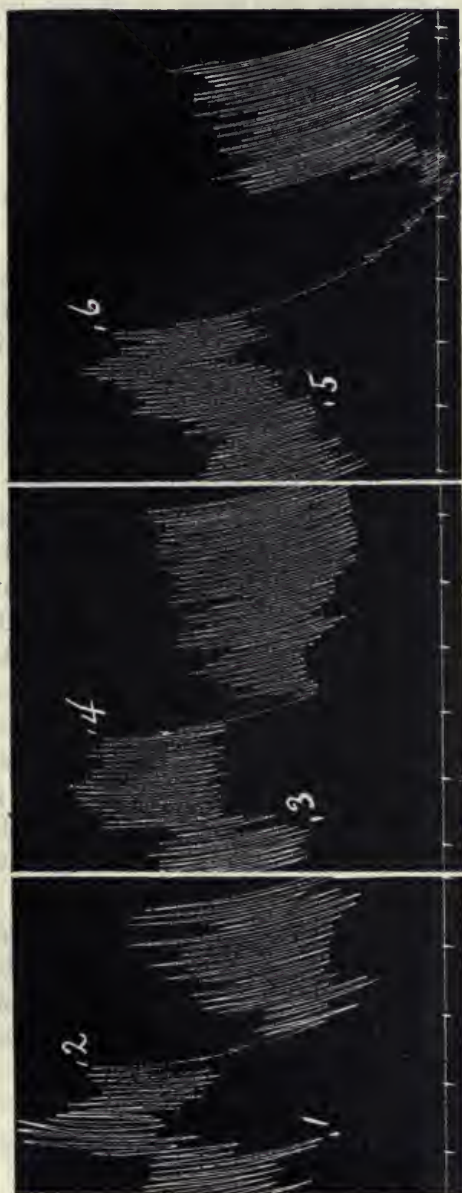


FIG. 2. INTESTINE TRACINGS

Bloods from cat 214. At 1, 3, and 5 Ringer was replaced by jugular blood and this at 2 by the second adrenal specimen (collected before carbonate injection); at 4 by the fourth adrenal specimen (collected three minutes after carbonate injection); at 6 by the sixth adrenal specimen (collected one hour after carbonate injection). All the bloods were diluted with 3 volumes Ringer. Time trace half minutes. (Reduced to two-thirds.)

- 12.20 p.m. Third adrenal specimen, 2.55 grams in 60 seconds (2.55 grams per minute). Fourth adrenal specimen, 8.45 grams in 240 seconds (2.11 grams per minute).
- 12.40 p.m. Fifth adrenal specimen, 2.3 grams in 60 seconds (2.3 grams per minute). Sixth adrenal specimen, 8.85 grams in 240 seconds (2.21 grams per minute).

Indifferent blood obtained from the abdominal aorta. Combined weight of adrenals 0.496 gram.

The result was quite different. Although an effect was produced upon the respiration, no definite increase in the output of epinephrin was made out in any of the adrenal blood specimens.

The epinephrin assay showed that the second adrenal specimen (taken before carbonate injection) was stronger than 1:4,000,000, somewhat weaker than 1:3,000,000. It was taken at 1:3,200,000, corresponding to an output of 0.00045 mgm. per minute for the cat, or 0.0002 mgm. per kilogram per minute.

The fourth specimen (collected three minutes after the beginning of the carbonate injection) had a concentration not very different from that of the second. It was assayed at 1:3,500,000, equivalent to an output of 0.0006 mgm. per minute for the cat, or 0.00027 mgm. per kilogram per minute. The sixth specimen (collected twenty-three minutes after injection of carbonate) had the same concentration as the fourth, corresponding also to an output of 0.00027 mgm. per kilogram per minute. The fourth specimen showed a slightly increased output, but the change, if present, was not clearly beyond the limits of error of such determinations.

In the next experiment (cat 213) three times as much carbonate as was used in cat 214 was injected, but in the form of 30 cc. of the 5 per cent solution, in three injections of 10 cc. each into the jugular vein, administered over a period of ten minutes.

Condensed protocol. Cat 213, male; weight, 2.675 kgm.

Anesthetized with urethane. Obtained indifferent blood from jugular. Made cava pocket.

- 11.10 a.m. Collected adrenal blood. First specimen, 3.75 grams in 60 seconds (3.75 grams per minute). Second specimen, 9.8 grams in 180 seconds (3.3 grams per minute).

- 11.15 a.m. Started artificial respiration and kept it up throughout rest of experiment.
- 11.20 a.m. to 11.30 a.m. 30 cc. of 5 per cent sodium carbonate injected into external jugular vein; in 10 cc. injections; with last injection the pupils became wide and cat gasping, but pupils soon came back to previous condition.
- 11.33 a.m. Third adrenal specimen, 2.55 grams in 60 seconds (2.55 grams per minute). Fourth adrenal specimen, 7.9 grams in 180 seconds (2.63 grams per minute).
- 12.07 p.m. Fifth adrenal specimen, 1.75 grams in 60 seconds (1.75 grams per minute). Sixth adrenal specimen, 4.6 grams in 180 seconds (1.53 grams per minute).

Obtained additional indifferent blood from jugular. Combined weight of adrenals 0.468 gram.

The second adrenal specimen (collected before injection of carbonate) had a concentration of 1:3,500,000, equivalent to an output of epinephrin of 0.00095 mgm. per minute for the cat, or 0.00035 mgm. per kilogram per minute. The fourth specimen (collected four minutes after the end of the carbonate injection) was slightly stronger than the second, but the flow was correspondingly smaller. Taking the fourth specimen at 1:3,000,000, we get 0.0009 mgm. for the cat, or 0.00033 mgm. per kilogram per minute. The sixth adrenal specimen (collected thirty-eight minutes after the end of the carbonate injection) assayed at 1:1,000,000 epinephrin, corresponding to an output of 0.0015 mgm. per minute for the cat, or 0.00055 mgm. per kilogram per minute.

In this experiment, then, no increase in the epinephrin output could be demonstrated in the specimen taken soon after the administration of carbonate. A remote specimen (the sixth) collected thirty-eight minutes after the carbonate injection gave an increase in the output of about 50 per cent.

We do not, of course, conclude that the reaction of the blood or the amount of the alkali reserve exerts no influence upon the rate of liberation (or of formation) of epinephrin. The available methods could not possibly detect small changes. For this reason it was not considered worth while to determine the alteration

in the H-ion concentration of the blood produced by the administration of carbonate. Neither did it enter into our plan to extend the investigation to concentrated solutions of other sodium salts or of dextrose or similar substances. Our object was sufficiently attained when the seemingly anomalous results which were the starting point of the investigation had been traced to the entrance of small quantities of the concentrated sodium carbonate into the circulation, and when the action upon the epinephrin output had been correlated by its time relations and in other ways with a general stimulating action of the solutions upon the bulbo-spinal centres. As the mechanism of the similar, but more intense, much more prolonged and apparently more interesting and important reactions produced by strychnine was being studied in detail (4), (5), it was not judged worth while, at least at present, to define more exactly the seat and mode of action of the concentrated salt solution. Some indications were obtained that in addition to increasing the output of epinephrin, injection of concentrated sodium carbonate solution may at a certain stage produce the opposite effect. But with the relatively small number of experiments performed it was not possible, as in the case of strychnine, to be sure that this action necessarily preceded the stimulating action, nor were the indications of an inhibitory or depressant action so clear. It is, of course, possible that the remote action of the carbonate in virtue of the alkalosis or through some other toxic effect upon the organism, may cause a diminution in the epinephrin output unconnected with the effects of the primary stimulation of the nervous mechanism.

One experiment was made to test whether any marked effect was produced by intravenous injection of concentrated sodium carbonate solution upon the epinephrin store of the adrenals.

Condensed protocol. Cat 218; female; weight, 3.4 kgm.

10.00 a.m. Anesthetized with urethane.

11.20 a.m. Excised right adrenal.

11.25 a.m. Blood pressure 146 mm. Hg.

11.26 a.m. Injected 2 cc. sodium carbonate solution (half to three-quarters saturated), intravenously; respiration stopped; started artificial respiration, which was kept up throughout the rest of the experiment. The blood pressure fell gradually during 4 minutes to 72 mm.; then suddenly began to ascend rapidly, reaching a maximum of 235 mm. It remained at about 218 mm. for 3 minutes then again suddenly mounted to about 280 mm. where it remained for 3 to 4 minutes, then gradually fell to 120 mm.

12.00 m. Excised left adrenal; blood pressure 98 mm.

12.05 p.m. Injected 2 cc. carbonate solution—intravenously. Blood pressure at once fell from 94 to 85 mm., then suddenly rose to a maximum of 160 mm.; then gradually fell to 90 mm. in 4 minutes.

12.15 p.m. Blood pressure again mounted, without apparent cause, reaching a maximum of 140 mm.; then gradually fell to 60 mm. in about 5 minutes. Within the next few minutes the pressure fell to zero, and the cat died.

Right adrenal weighed 0.211 gram and contained 0.28 mgm. epinephrin. Left adrenal weighed 0.206 gram and contained 0.21 mgm. epinephrin.

As it was not practicable to use a non-anesthetized animal, one adrenal was excised from a urethanized cat and the carbonate injected into the jugular vein. An enormous rise of blood pressure (to 280 mm. of mercury), ensued after a preliminary fall very much as in cat 214, the chief difference being that the initial minor rise of pressure was absent and that the period which elapsed between the injection and the commencement of the main rise was much longer. The increase in the blood pressure was also more sustained. The increased output of epinephrin during this period did not make any serious inroad upon the epinephrin store of the remaining adrenal. For when it was excised forty minutes later than the first, the store was not diminished more than is usually seen in such an experiment under urethane anesthesia without carbonate injection. The interval between the removal of the two adrenals was purposely made relatively short, so that the diminution associated with the

experimental conditions might not mask completely any more rapidly developed effect due to the carbonate.

While it is not possible from this experiment to determine whether an increased liberation of epinephrin from the one adrenal remaining took any sensible share in the great rise of blood pressure, it was shown clearly by injecting carbonate after removal of the second adrenal that a good rise of pressure was obtained in the absence of epinephrin liberation. Naturally the absolute amount of the rise was less than after the previous injection, as the condition of the animal, of course, had deteriorated. Our experiments on strychnine indicate that even the greater and much more sustained increase in epinephrin output produced by that drug can play but a minor rôle in the increase of arterial pressure.

A point of technique of some importance follows from the above observations. Concentrated solutions of salts must not be employed in the connections of the artery with a mercurial manometer if blood pressure tracings are being taken in experiments on the epinephrin output. If such substances are used, and the conditions of the experiment involve considerable changes of blood pressure, as in experimental shock, e.g., the greatest care must be taken that none of the solution passes into the artery. We abandoned carbonate as soon as our suspicions were confirmed and reverted to sodium citrate solutions (2 per cent). Pains are taken even with the citrate, to prevent any of the solution from entering the circulation, by adjusting the pressure in the manometer from time to time so that it is always a little below the arterial pressure.

SUMMARY

1. Intravascular injection of small volumes of concentrated salt solutions (sodium carbonate) causes a temporary increase in the rate of liberation of epinephrin from the adrenals.
2. This increase is presumably due to stimulation of the nervous mechanism which governs the epinephrin output since it is accompanied by symptoms of a general excitation of the

bulbo-spinal centers, and is not obtained, or only in a minor degree, when even larger quantities of the carbonate are injected in more dilute form.

3. In experiments on epinephrin output, it is not advisable to use concentrated solutions of salts in tubes connecting an artery with a mercurial manometer.

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THE ACTION OF DRUGS ON THE OUTPUT OF EPINEPHRIN FROM THE ADRENALS

III. NICOTINE

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INTRODUCTION

Of the few statements in the literature as to the action of nicotine upon the output of epinephrin, none so far as we are aware, contains any quantitative data whatever. Nor is anyone of them based upon a method capable of yielding direct and unequivocal qualitative evidence. The best investigation is that of Dale and Laidlaw (1). But they merely observed that certain reactions which are elicited by nicotine on the non-pregnant uterus of the cat and in the eye after removal of the superior cervical ganglion are modified when the experiment is made under such conditions that epinephrin can no longer reach these structures from the adrenals. They explain the difference by the hypothesis that the nicotine action is in part due to a stimulation of the adrenals to increased liberation of epinephrin. We believe that indirect evidence of this kind, as has been set forth in another paper on the action of strychnine upon the epinephrin output (2), is valuable when it corroborates the results of more direct methods, but that when standing alone it must be interpreted with great care. For example, it would not be possible in these experiments to know whether as a consequence of the action of the drug on the circulation a larger amount of epinephrin per minute or a greater concentration of it might not be supplied to the uterus or the eye without any change whatever having occurred in the rate of discharge. It is scarcely neces-

sary to add that in any case such observations, if it be granted that they indicate a stimulating effect of nicotine upon the epinephrin output, do not afford the means of measuring the amount of the increase.

These remarks are not intended as a criticism of these interesting and suggestive experiments, they are meant merely to point out the limitations of such indirect methods.

Gley's experiment (3) on the effect of nicotine upon the blood pressure before and after excision of the adrenals in animals with the spinal cord destroyed, besides being also a purely qualitative experiment, loses most of its weight by proving too much. He gives a figure showing a rise of pressure from less than 30 mm. to 140 mm. of mercury, following injection of 10 mgm. of nicotine into a 9 kgm. dog with intact adrenals, whereas after excision of the adrenals, the same dose of nicotine only caused a rise from 24 mm. to about 30 mm. He concludes that practically the whole pressor effect of nicotine after destruction of the bulbo-spinal centres is due to the increase produced by it in the output of epinephrin. But he did not determine whether in the second part of his experiment a given dose of epinephrin would produce as great an effect as in the first part, and we know that great changes can occur in the magnitude of the response to a given quantity of epinephrin in the course of an experiment involving such an operation, even when the splanchnics have not been injured in the adrenalectomy. Five hundred cubic centimeters of Locke's solution had been injected to keep up the pressure before the first dose of nicotine was administered, but no mention is made of any solution having been injected after adrenalectomy although the pressure was still lower. We have seen that injection of Ringer's solution under conditions of low blood pressure may markedly augment the pressor effect of epinephrin, and no doubt this would be true also of vasomotor stimulation.

For example, 250 cc. of Ringer's solution was injected into a 9 kgm. dog when the blood pressure had sunk to 27 mm. of mercury. The heart's action improved and the blood pressure rose somewhat (to 50 mm. of mercury), and remained steady at about this level for a con-

siderable time. The effect on the blood pressure of 0.5 cc. of a 1:33,000 solution of adrenalin introduced into the jugular vein before the injection of the Ringer's solution was no greater than the effect of 0.5 cc. of a 1:130,000 after the injection. In another observation 0.5 cc. of 1:65,000 adrenalin before the injection of the Ringer's solution did not give as great an effect as 1:260,000 after the injection. As in all our papers, the concentration of adrenalin solutions is expressed in terms of the base, determined colorimetrically, by the method of Folin, Cannon and Denis (4).

Langley (5) obtained an excellent rise of pressure when nicotine was injected after the adrenals had been tied off from the circulation. In some of our own experiments, the nicotine injection was made while the adrenal blood was being collected, yet a good increase of pressure was seen. Here, of course, the bulbo-spinal centers may have participated in the action. And it may be assumed that if nicotine stimulates the ganglion cells on the path of the adrenal secretory fibres, or in the absence of ganglion cells on the path of these fibres (6) possibly some part of the medullary cells themselves, an increased epinephrin output may contribute to the rise of pressure. But all the evidence goes to show that even when the output has been proved by direct methods to be considerably augmented and the adrenal blood after being collected for a time is suddenly released the epinephrin factor in the increase of pressure is far subordinate to the vasomotor factor. And it would appear in the highest degree improbable that the excitation of the ganglion cells on the course of the vasomotors should be a negligible factor in the vasoconstriction in comparison with the excitation of the adrenal secretory fibres.

Cannon, Aub and Binger (7) conclude that "injection of nicotine in small amounts (3.5 to 7.5 mgm. in cats) results in augmented adrenal secretion." It is impossible, however, by the method used (collection of blood by a catheter from the inferior cava above the level of the adrenal veins) to arrive at any conclusion as to the effect of the drug upon the rate of output of epinephrin. For changes in the rate of the blood flow were not taken account of, and it has been already pointed out more than

once (2), although this would scarcely seem necessary, that to determine the output of epinephrin by applying blood to such objects as intestine strips or segments, two quantities must be measured, the concentration of epinephrin in the blood coming from the adrenals and the amount of blood passing through them in unit of time. That the concentration of epinephrin in the adrenal vein blood and, therefore, in the cava blood above the adrenal veins may be increased at some stage after the injection of such doses of nicotine is certain owing to the diminution in the rate of blood flow associated with the marked and prolonged fall of blood pressure succeeding the very brief rise. But such an increase of concentration is always observed when the blood flow in the cava is slowed from any cause whatever, provided that the epinephrin output continues unchanged or is diminished less than the rate of the blood flow.

Technique. Our experiments were all made upon cats. The technique has been described sufficiently in the first paper of this series (2). Direct determinations upon rabbit intestine (and uterus) segments of the epinephrin concentration in specimens of pure adrenal blood collected at a measured rate were chiefly relied upon. Corroborative evidence was obtained by a method of auto-assay, by means of the blood pressure reactions produced by adrenal blood when collected in a cava pocket for a given time and then released into the circulation.

Comparison of the (denervated) eye reactions in a normal cat with those in a cat whose epinephrin discharge had been interfered with by previous excision of one adrenal and denervation of the other, was also employed as a corroborative method, although it was recognized that the evidence afforded by this method was distinctly inferior to that yielded by the other two, especially the first mentioned, and far more difficult of interpretation.

One experiment was made to determine whether nicotine produced a demonstrable effect upon the epinephrin store of the adrenals.

EXPERIMENTS WITH INTRAVENOUS INJECTION OF NICOTINE AND ASSAY OF ADRENAL BLOOD ON RABBIT SEGMENTS

The experiments yielded strikingly clear and consistent results. The predominant action of nicotine upon the epinephrin output was shown to be a depressant or paralysing action. This

effect is easy to detect, begins early and lasts a relatively long time, its duration naturally depending upon the dose. Quantitatively the rate of output may be diminished, when the paralysing action is at its height, to a third, a quarter or a fifth of the normal or initial output before the administration of the drug, or no epinephrin at all may be detected with certainty by the test objects. Gradually recovery ensues and the rate increases, but it may or may not (in these acute experiments) regain the original amount.

This stage of paralysis is preceded by a very brief period during which the epinephrin output is more or less markedly augmented, according to the dose and other conditions. Blood pressure tracings taken while the adrenal blood specimens were being collected, showed that the augmentation of the output coincided approximately with the period of increased blood pressure, and the diminished output with the much longer period during which the blood pressure remained below the original level. The diminution in the output was not due directly to the lowered blood pressure. For we have abundant evidence that with still lower pressures in the absence of nicotine, epinephrin continues to be liberated for long periods at the initial rate before the blood pressure was lowered. Also the abruptness with which the preliminary augmentation of the output gives place to the maximum diminution indicates that the initial stimulation has been quickly overborne by paralysis or depression just as in the case of the vasomotor mechanism.

It is to be presumed that both of these actions like the similar effects upon the efferent vasomotor paths are exerted upon sympathetic ganglion cells intercalated in the efferent secretory path of the adrenals (but see Elliott (6)).

With the smallest doses employed (about 0.1 mgm. of nicotine per kilogram of bodyweight) the depressant effect upon the epinephrin output was clearly demonstrated. None of the adrenal blood samples collected after these small doses of the poison, not even the samples taken immediately after its injection, corresponded to an output, calculated for the whole sample, greater than the original output before the nicotine injection. It was

shown, however, that this was simply due to the fact that the depressant action so quickly succeeded the stimulation that when the collection of the first sample after the nicotine lasted only one minute, the augmentation, which, of course, was less than with larger doses and continued only for a fraction of a minute, was totally masked by the diminished output during the rest of the minute.

In the first two experiments to be quoted (cats 281 and 283), although the dose was relatively large, we completely missed the transient preliminary augmentation of the output because we did not as yet know that it was so brief that it was necessary to cut down the interval between the nicotine injection and the collection of the adrenal blood to a minimum.

Condensed protocol. Cat 281; male; weight, 4.37 kgm.

Anesthetized with urethane. Obtained indifferent blood from the jugular. Made cava pocket. Collected adrenal blood.

11.45 a.m. First specimen, 2.1 grams in 30 seconds (4.2 grams per minute).

11.45½ a.m. Second specimen, 7.8 grams in 120 seconds (3.9 grams per minute).

11.54 a.m. Injected 2.5 mgm. nicotine intravenously.¹

11.56 a.m. Third adrenal specimen, 2.6 grams in 30 seconds (5.2 grams per minute).

11.56½ a.m. Fourth adrenal specimen, 7.0 grams in 120 seconds (3.5 grams per minute).

12.10 p.m. Fifth adrenal specimen, 1.9 grams in 30 seconds (3.8 grams per minute).

12.10½ a.m. Sixth adrenal specimen, 7.35 grams in 120 seconds (3.7 grams per minute).

12.15 p.m. Injected 5 mgms. nicotine intravenously.

12.17 p.m. Seventh adrenal specimen, 1.8 grams in 30 seconds (3.6 grams per minute).

12.17½ p.m. Eighth adrenal specimen, 4 grams in 150 seconds (1.6 grams per minute).

Obtained another specimen of venous blood. Combined weight of adrenals 0.553 gram.

¹ As in all of the experiments, the nicotine was injected in a 1:200 solution.

It is impossible to reproduce a sufficient number of tracings to illustrate adequately the epinephrin assays in any of the experiments. In figure 1 are given tracings showing the relative effects produced on a rabbit intestine segment by the three most important adrenal blood specimens. As the blood flows were nearly

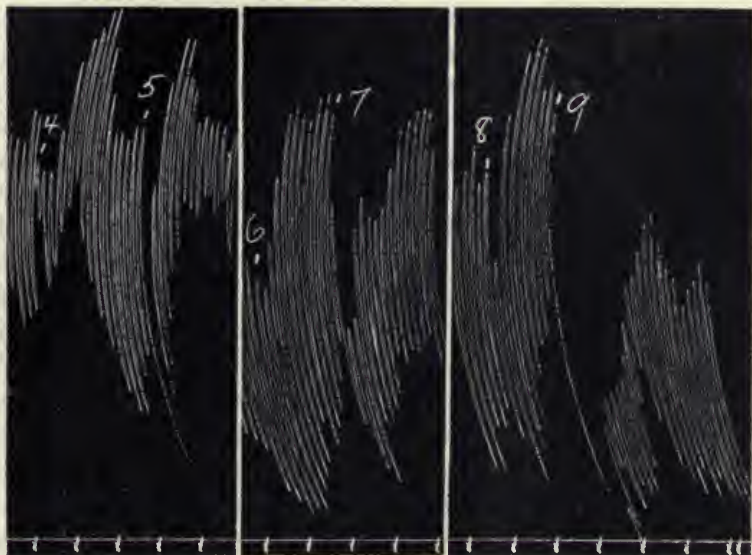


FIG. 1. INTESTINE TRACINGS. BLOODS FROM CAT 281

At 4, 6 and 8 Ringer was replaced by jugular blood and this at 5 by the fourth adrenal specimen (collected two and one-half minutes after nicotine injection); at 7 by the sixth adrenal specimen (collected sixteen and one-half minutes after injection of nicotine); at 9 by the second adrenal specimen (collected before injection of nicotine). All the bloods were diluted with three volumes Ringer.* (Reduced to two-thirds.)

* In all the intestine and uterus tracings time is marked in half minutes, in the blood pressure tracings in ten second intervals.

equal in these specimens, even the qualitative comparison of the tracings shows that nicotine produced a marked diminution in the epinephrin output. It was confirmed by uterus tracings that the sixth specimen was stronger than the fourth and the second stronger than the eighth. The detailed assay showed

that the second specimen, collected before injection of nicotine, was much stronger than 1:5,700,000 adrenalin, weaker than 1:3,000,000, not far from 1:4,300,000, probably slightly weaker. Taking it at 1:4,500,000, we get an output of 0.00087 mgm. per minute for the cat, or 0.0002 mgm. per kilogram per minute. The fourth specimen, whose collection was begun two and one-half minutes after the nicotine injection, was much weaker than the second (fig. 1, observations 5 and 9) although the blood flow was somewhat less in the fourth. Obviously, the output must have been greatly diminished at the time the fourth specimen was collected. The fourth specimen was found to be weaker than 1:11,400,000 adrenalin, somewhat weaker than 1:14,300,000. Taking it at 1:15,000,000, we get 0.00023 mgm. per minute for the cat, or 0.00005 mgm. per kilogram per minute, only one-fourth of the output before the administration of nicotine.

It must be remembered that if the transient preliminary increase in the output was present in this experiment, as is likely, seeing that we have never missed it, since we have known when to look for it, even with a dose relatively smaller than that used in this experiment, some of the epinephrin belonging to the period of excitation might have been included in the fourth specimen at the beginning of its collection. The reduction in the output during the greater part of the period of collection would then be much greater than that calculated from the assay. The sixth specimen (collected sixteen to seventeen minutes after nicotine) had a much smaller concentration of epinephrin than the second (fig. 1, observations 7 and 9), although the flow was slightly less. The depressant effect was, therefore, still quite marked, but some recovery had taken place since the sixth specimen was stronger than the fourth. It was demonstrated by three separate sets of observations (one set is reproduced in figure 1) that the sixth specimen was intermediate in concentration between the second and the fourth. It was the same whether the venous blood containing nicotine, collected at the end of the experiment, or the venous blood drawn before injection of nicotine, was employed as indifferent blood. In our assays of adrenal blood specimens collected after nicotine, we always test them

against nicotine indifferent blood as well as against nicotine-free indifferent blood. The assay showed that the sixth specimen was weaker than 1:7,100,000, weaker than 1:8,570,000, stronger than 1:11,400,000. It was finally taken at 1:9,500,000, corresponding to an output of 0.0004 mgm. per minute for the cat, or 0.00009 mgm. per kilogram per minute, nearly half the rate before the injection of nicotine. Accordingly, the recovery in the output sixteen or seventeen minutes after the injection of nicotine was substantial, but it was still much below the original output.

After the collection of the sixth specimen another larger dose of nicotine was administered. The eighth adrenal specimen was obtained two to three minutes thereafter. It was found to have about the same concentration of epinephrin as the second specimen, viz., 1:4,500,000. But as the blood flow during collection of the eighth was less than half as great as during collection of the second, the output per minute was much less, 0.00035 mgm. per minute for the cat, or 0.00008 mgm. per kilogram per minute. If the second injection of nicotine caused any excitation of the epinephrin secretion at this time, which we may assume from other experiments was the case, the effect was over before the eighth specimen was obtained, and only the depressant action was in evidence.

In the next experiment (cat 283) a much larger dose of nicotine was employed in the hope of obtaining the *increase* in epinephrin output which was the only action mentioned in the scanty literature of the subject.

Condensed protocol. Cat 283; male: weight, 3.38 kgm

Anesthetized with ether. Obtained indifferent blood from jugular. Made cava pocket. Collected adrenal blood.

11.55 a.m. First specimen, 1.55 grams in 30 seconds (3 grams per minute).

11.55½ a.m. Second specimen, 5.55 grams in 120 seconds (2.8 grams per minute). Blood pressure at the end of collection of second specimen was 94 mm. mercury; (fig. 2, observation 3).

- 12.05 p.m. Injected 7.5 mgm. nicotine intravenously (fig. 2, observations 4 and 5); started artificial respiration (observation 6).
- 12.07 p.m. Third adrenal specimen, 0.7 gram in 60 seconds (0.7 gram per minute).
- 12.08 p.m. Fourth adrenal specimen, 2.55 grams in 420 seconds (0.37 gram per minute). Blood pressure during collection of third specimen was 34 mm. mercury (fig. 2, observation 7); blood pressure during collection of fourth specimen was 26 mm. mercury (fig. 2, observation 8).
- 12.25 p.m. Fifth adrenal specimen, 0.3 gram in 60 seconds (0.3 gram per minute).
- 12.26 p.m. Sixth adrenal specimen, 1.2 grams in 300 seconds (0.24 gram per minute). Blood pressure during collection of fifth specimen was 24 mm. mercury (fig. 2, observation 9); blood pressure during collection of sixth specimen was 22 mm. mercury (fig. 2, observation 10).

Obtained another specimen of venous blood. Combined weight of adrenals 0.33 gram.

There was, of course, in the blood pressure tracings of all the experiments a certain amount of lost time in making the marks, so that each should be moved a second or two to the left. It is still more important to remember that the signal marks, indicating the beginning or end of collection of the specimen, must all be shifted to the left in order to really correspond with the blood pressure curve, because of the dead space between the adrenal veins and the open end of the cannula. The number of seconds to be allowed for this will, of course, depend upon the rate of the blood flow. This correction cannot be exactly made since the dead space is not exactly known. In our experiments, it varied from one-half to one cc. according to the size of the cat and was somewhat less for the later than for the earlier specimens, since in putting in fresh cannulae, the cava pocket of course grew shorter.

Another factor which prevents an exact correspondence of the blood pressure curve with the curve of epinephrin output, is that although the latency of the adrenal response to stimulation of the secretory nerves, as shown in a previous paper (8) is exceedingly short, it cannot be supposed that epinephrin mobilized in response to stimulation of the secretory nerves will as quickly reach the blood as the arterioles will contract to vasomotor stimulation.

Figure 2 shows the points on the blood pressure tracing at which the various adrenal blood specimens were collected. Although the interval between the injection of the nicotine and the beginning of collection of the fourth specimen was only three minutes the period of increased blood pressure was long since over, and as we found out later, it was useless to look at this time for an increased epinephrin output.

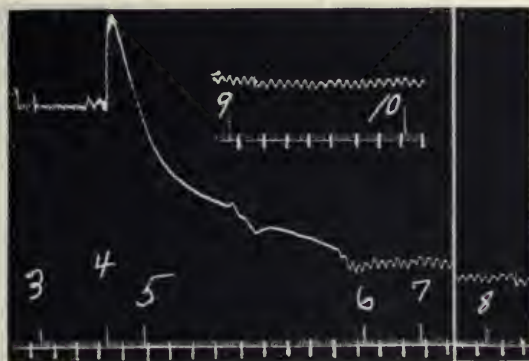


FIG. 2. BLOOD PRESSURE TRACING. CAT 283

3 marks the end of collection of the second adrenal specimen; 4 and 5 beginning and end of nicotine injection; at 6 artificial respiration was begun; 7 beginning of collection of third adrenal specimen; 8 end of collection of fourth adrenal specimen; 9 beginning of collection of fifth adrenal specimen; 10 end of collection of sixth adrenal specimen. Line of zero pressure same as time trace. (Reduced to two-thirds.)

The second specimen (taken before injection of nicotine) was found to be decidedly weaker than 1:5,300,000, and somewhat stronger than 1:8,000,000. It was taken at 1:7,000,000, corresponding to an output of 0.0004 mgm. per minute for the cat, or 0.00012 mgm. per kilogram per minute.

The fourth adrenal blood specimen (collected three minutes after the administration of nicotine) had a much greater concentration of epinephrin than the second (fig. 3). Since, however, the blood flow during collection of the fourth specimen was only one-eighth as great as during collection of the second, the

mere qualitative comparison of the inhibitory effects produced on the intestine segment yields no information whatever as to any change in the rate of output. As a matter of fact, the assay proved that the fourth specimen, (which was much weaker than 1:1,000,000, weaker than 1:1,300,000, weaker than 1:2,000,000, stronger than 1:2,700,000 and was finally taken at 1:2,400,000), gave an output of only 0.00015 mgm. per minute for the cat, or

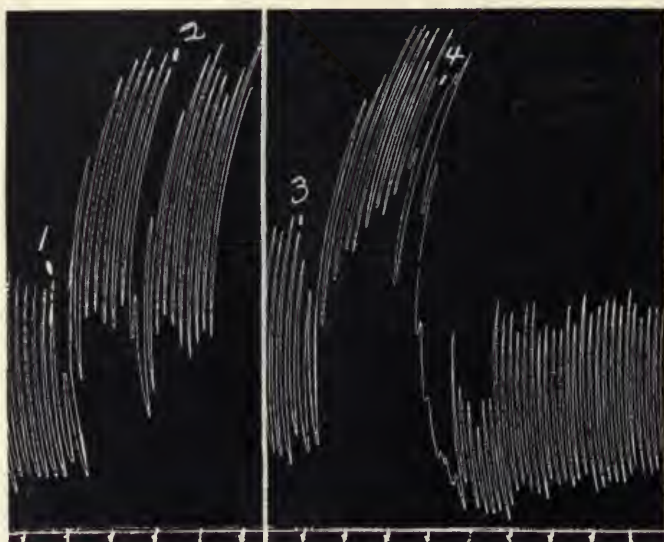


FIG. 3. INTESTINE TRACINGS. BLOOD FROM CAT 283

At 1 and 3 Ringer was replaced by jugular blood and this at 2 by the second adrenal specimen (collected before injection of nicotine); at 4 by the fourth adrenal specimen (collected three minutes after injection of nicotine). All the bloods were diluted with three volumes Ringer. (Reduced to two-thirds.)

0.000045 mgm. per kilogram per minute, only about one-third of the output before nicotine. This was not because the blood flow was so small that the concentration of epinephrin had reached the possible maximum. It was far below the normal maximum, and, indeed, it will be shown presently that one of the characteristics of the nicotine stimulation of the epinephrin secretion, which distinguishes it from the stimulation due to strychnine,

nine, is that the possible normal maximum concentration can be much exceeded. The depressant effect of the nicotine was, therefore, distinctly in evidence at this time, not only as regards the vasomotor, but as regards the adrenal secretory fibres.

The sixth adrenal specimen (taken twenty-one minutes after nicotine) was found to be much weaker than 1:1,300,000, weaker than 1:2,000,000, and approximately equal to 1:2,700,000, corresponding to an output of 0.00009 mgm. per minute for the cat, or 0.000025 mgm. per kilogram per minute, little more than one-fifth of the output before nicotine. Figure 4 shows that the sixth specimen was somewhat weaker than the fourth.

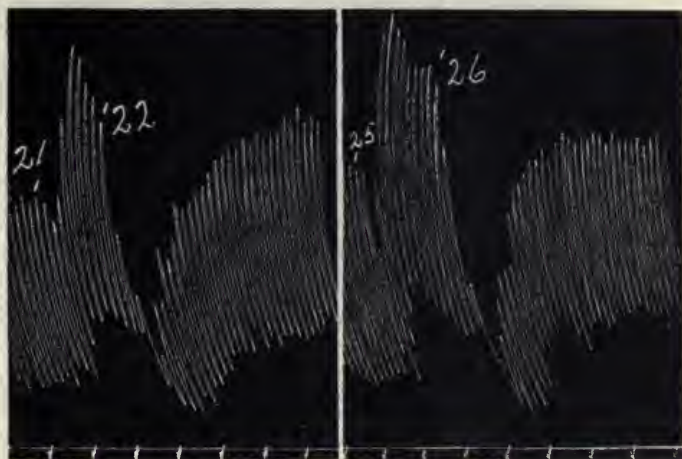


FIG. 4. INTESTINE TRACINGS. BLOOD FROM CAT 283

At 21 and 25 Ringer was replaced by venous blood collected after the injection of nicotine; and this at 22 by the fourth adrenal specimen (collected three minutes after injection of nicotine); at 26 by the sixth adrenal specimen (collected twenty minutes after the injection of nicotine). All the bloods were diluted with three volumes Ringer. (Reduced to two-thirds.)

Condensed protocol. Cat 284; male; weight, 2.85 kgm.

Anesthetized with ether. Obtained indifferent blood from jugular. Made cava pocket. Collected adrenal blood.

11.17 a.m. First specimen, 1.2 grams in 30 seconds (2.4 grams per minute).

- 11.17 $\frac{1}{2}$ a.m. Second specimen, 4.0 grams in 120 seconds (2 grams per minute). Blood pressure 100 mm. mercury (fig. 5, observation 1).
- 11.25 a.m. Injected 2 mgm. nicotine intravenously (fig. 5, observations 2 and 3).
- 11.25 $\frac{1}{2}$ a.m. Third adrenal specimen, 0.9 gram in 30 seconds (1.8 grams per minute).
- 11.26 a.m. Fourth adrenal specimen, 1.6 grams in 120 seconds (0.8 gram per minute). Blood pressure at beginning of collection of third specimen was 114 mm. mercury (fig. 5, observation 4); blood pressure during collection of fourth specimen was falling. The pressure at the beginning of the collection was 52 mm. and at the end of the collection was 20 mm. mercury (fig. 5, observation 5).
- 11.40 a.m. Fifth adrenal specimen, 0.75 gram in 30 seconds (1.5 grams per minute).
- 11.40 $\frac{1}{2}$ a.m. Sixth adrenal specimen, 3.9 grams in 180 seconds (1.3 grams per minute). Blood pressure during collection of fifth specimen was 70 mm. mercury (fig. 5, observation 8); blood pressure during collection of sixth specimen was 66 mm. mercury (fig. 5, observation 9).

Obtained another specimen of venous blood. Combined weight of adrenals 0.502 gram.

In the above experiment (cat 284) adrenal blood was collected almost immediately after completion of the injection of a much smaller dose of nicotine than in the last experiment, with the object of catching, if possible, a sample which would show an increased output before the paralysis came on. The expectation was realised. Figure 5 shows the position on the blood pressure curve of the various adrenal specimens. The third adrenal specimen (collected about one-half minute after the nicotine injection) had by far the greatest concentration of epinephrin of all the specimens (fig. 6, observation 34). It was proved by the assay to be much stronger than 1:660,000 adrenalin, stronger than 1:330,000 adrenalin (fig. 6, observations 30 and 36). Several other observations, not reproduced, showed that it was fully as strong as 1:300,000. But taking it at 1:300,000, we get for the

output of epinephrin, at this time, 0.006 mgm. per minute for the cat, or 0.002 mgm. per kilogram per minute. In as much as the time at which collection of a specimen begins nominally must be corrected for the time required to fill up the dead space in the cava and cannula at the given rate of flow, the blood of the third specimen really began to leave the adrenals a very few seconds after injection of the nicotine.

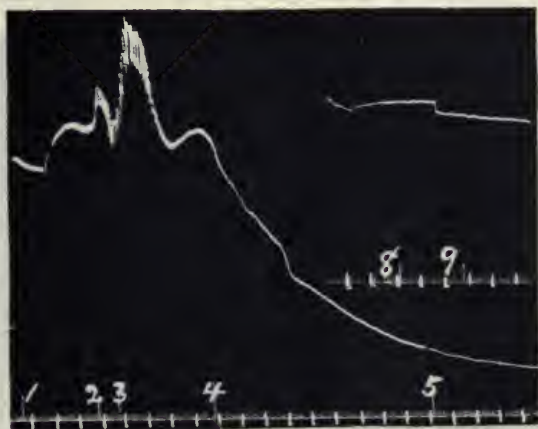


FIG. 5. BLOOD PRESSURE TRACING. CAT 284

1, blood pressure during collection of adrenal blood before nicotine injection; 2 to 3, injection of nicotine; 4, beginning of collection of third adrenal specimen; 5, during collection of the fourth adrenal specimen; 8, beginning of collection of fifth adrenal specimen; 9, during collection of sixth adrenal specimen. Line of zero pressure corresponds with the time trace and is moved up 15 mm. and the figure then reduced to two-thirds.

The second adrenal blood specimen (collected before injection of nicotine) was assayed at 1:7,000,000, corresponding to an output of 0.0003 mgm. per minute for the animal, or 0.0001 mgm. per kilogram per minute. The third specimen, therefore, represents a rate of output not less than twenty times the original output before nicotine. The sixth adrenal specimen (collected a quarter of an hour after the administration of nicotine when the blood pressure had recovered to 100 mm. of mercury), had a somewhat greater concentration of epinephrin than the second

specimen (fig. 7, observations 2 and 6). It was assayed at 1:6,000,000, corresponding to an output of 0.0002 mgm. per minute for the cat, or 0.00007 mgm. per kilogram per minute. The initial output had not been quite recovered at this time.

The fourth adrenal blood specimen (collected one minute to three minutes after the nicotine injection, while the blood pressure was falling to its minimum level) was found to be much stronger than either the sixth or the second (fig. 7). The blood flow, of

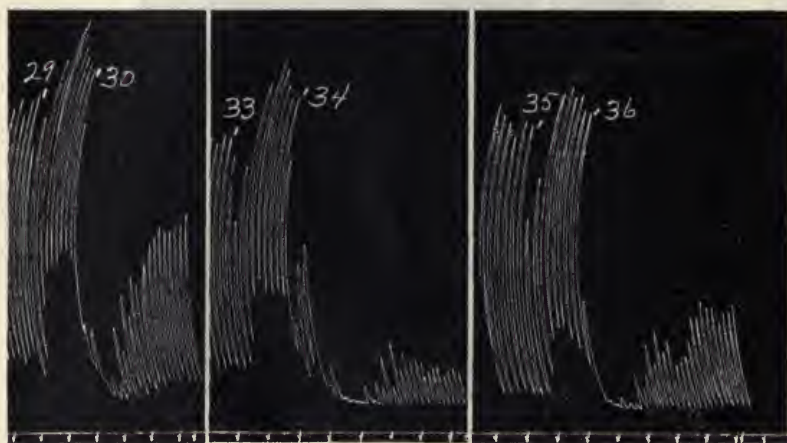


FIG. 6. INTESTINE TRACINGS. BLOODS FROM CAT 284

At 29, 33 and 35 Ringer was replaced by venous blood collected after injection of nicotine; and this at 30 by venous blood to which was added adrenalin to make a concentration of 1:660,000; at 34 by the third adrenal specimen (collected immediately after injection of nicotine); at 36 by venous blood to which was added adrenalin to make a concentration of 1:330,000. All the bloods were diluted with three volumes Ringer (the adrenalin bloods after adding the adrenalin). (Reduced to one-half.)

course, owing to the low blood pressure was much less. The inhibition of the intestine segment produced by the fourth specimen was decidedly less than that caused by the third. The difference was greater than would appear from comparison of figure 8, observation 24, and figure 6, observation 34, since the sensitiveness of the segment to epinephrin was proved to have diminished between observations 26 and 28 (not reproduced).

The assay showed that the fourth specimen was stronger than 1:930,000 (fig. 8, observations 24 and 26), and somewhat stronger than 1:660,000. Taking it at 1:600,000, we get 0.0013 mgm. per minute for the cat, or 0.00045 mgm. per kilogram per minute; four or five times the rate of the output before nicotine. It is practically certain that this relatively high output is due entirely to an overlapping of the period of excitation, which reached its maximum during collection of the third specimen, into the first part of the period of collection of the fourth. If the average rate of output for the third specimen continued for the first half

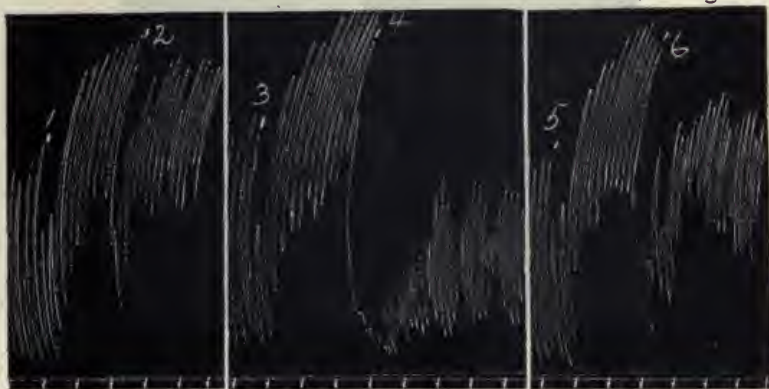


FIG. 7. INTESTINE TRACINGS. BLOODS FROM CAT 284

At 1, 3 and 5 Ringer was replaced by jugular blood and this at 2 by the second adrenal specimen (collected before injection of nicotine); at 4 by the fourth adrenal specimen (collected one minute after injection of nicotine); at 6 by the sixth adrenal specimen (collected fifteen minutes after injection of nicotine). All the bloods were diluted with three volumes Ringer. (Reduced to one-half.)

minute of collection of the fourth, this would account for the whole content of epinephrin in the fourth specimen even if none at all was liberated during the remaining one and one-half minutes. It must be remembered that owing to the "lag" entailed by the filling of the dead space, the real commencement of collection of the fourth specimen would be only a little more than one-half minute after the nicotine injection. From the evidence of other experiments it is almost certain that the paralysing

action of the drug would be fully developed during the latter part of the period of collection of the fourth specimen and would continue till it began to disappear as the pressure gradually rose.

The proportion of serum in the blood, determined by the hematocrite (after 34 minutes rotation at 4000 times a minute) was 52 per cent. The epinephrin concentration in the serum of the third specimen must, therefore, have been nearly 1:150,000. This is fully three times the possible normal maximum concen-

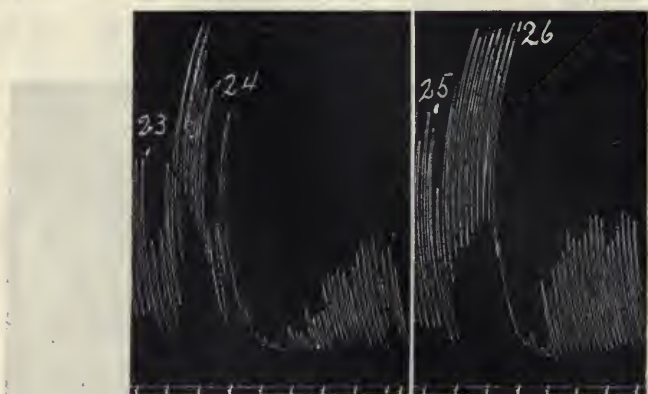


FIG. 8. INTESTINE TRACINGS. BLOODS FROM CAT 284

At 23 and 25 Ringer was replaced by venous blood (collected after injection of nicotine) and this at 24 by the fourth adrenal specimen (collected one minute after injection of nicotine); at 26 by venous blood to which was added adrenalin to make a concentration of 1:1,000,000. All the bloods were diluted with three volumes Ringer (the adrenalin blood after adding the adrenalin). (Reduced to one-half.)

tration as defined in the first paper of this series (2). It was there stated that in none of the strychnine experiments had the possible normal maximum concentration of epinephrin (about 1:500,000, as determined on rabbit segments) been exceeded even with the slowest flows and the greatest increases in the output. Apparently, then, the transient stimulation of the secretion caused by nicotine differs from the long lasting stimulation caused by strychnine, in that the former for the brief period for which it lasts can force up the normal maximum con-

centration while the latter cannot. If this distinction is well founded it is of considerable interest. For it indicates that the strychnine action is essentially a speeding up of the normal process presumably by an increase in the excitability of the central mechanism, whereas the nicotine action is an artificial and quite unphysiological stimulation of the sympathetic relay on the efferent path.

Condensed protocol. Cat 285, male, weight, 4.4 kgm.

Anesthetized with ether. Obtained indifferent blood from jugular. Made cava pocket. Collected adrenal blood.

11.40 a.m. First specimen, 2.6 grams in 30 seconds (5.2 grams per minute). Second specimen, 9.2 grams in 120 seconds (4.6 grams per minute). Blood pressure 146 mm. mercury (fig. 9, observation 3).

11.50 a.m. Injected 2 mgm. nicotine intravenously (fig. 9. observations 4 and 5).

11.50½ a.m. Third adrenal specimen, 1.35 grams in 30 seconds (2.7 grams per minute).

11.51 a.m. Fourth adrenal specimen, 2.05 grams in 120 seconds (1.03 grams per minute).

11.53 a.m. Fifth adrenal specimen, 3.35 grams in 360 seconds (0.56 gram per minute).

Blood pressure at the beginning of the collection of the third specimen was 116 mm. mercury (fig. 9. observation 6); at the beginning of the collection of the fourth specimen 70 mm. mercury (observation 7); at the beginning of the collection of the fifth specimen 27 mm. mercury (observation 8).

12.12 p.m. Sixth adrenal specimen, 1.8 grams in 30 seconds (3.6 grams per minute).

12.12½ p.m. Seventh adrenal specimen, 9.3 grams in 180 seconds (3.1 grams per minute).

Blood pressure during collection of the sixth specimen was 92 mm. mercury (fig. 9. observation 16); blood pressure during collection of the seventh specimen was 85 mm. mercury (fig. 9, observation 17).

Obtained another specimen of venous blood. Combined weight of adrenals 0.553 gram.

In the above experiment (cat 285) a still smaller dose of nicotine, reckoned on the bodyweight, was employed in order to see whether the stage of depression which cuts short the preliminary stimulation could thus be postponed, permitting a longer period of increased output. This was not found to be the case. In essentials the results were precisely the same as in the preceding experiment. The blood pressure curve followed exactly the same course and the changes in epinephrin output were roughly parallel to the blood pressure curve.

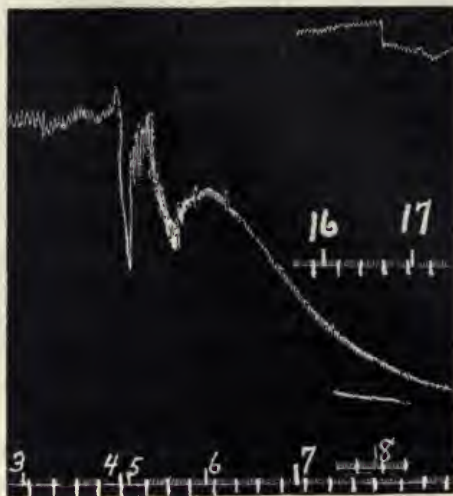


FIG. 9. BLOOD PRESSURE TRACING. CAT 285

3, Blood pressure during collection of second adrenal specimen (before nicotine injection); 4 to 5, injection of nicotine; 6, beginning of collection of third adrenal specimen; 7, beginning of collection of fourth adrenal specimen; 8, beginning of collection of fifth adrenal specimen; 16, end of collection of sixth adrenal specimen; 17, end of collection of seventh adrenal specimen. Line of zero pressure corresponds with the time trace. (Reduced to two-thirds.)

Figure 9 shows the points on the blood pressure curve at which the various adrenal blood specimens were collected. The second specimen, collected before injection of nicotine, produced a much smaller inhibition of the intestine segment than the fourth speci-

men, collected one minute after nicotine (fig. 10). Since the blood flow during collection of the fourth specimen was less than one-fourth of the flow during collection of the second, this difference would not of itself prove that the epinephrin output was increased for the fourth specimen. The assay on rabbit intestine (and uterus) segments showed, however, that this was the case.

The second specimen was found to be much weaker than 1:2,600,000 adrenalin, weaker than 1:4,000,000, weaker than 1:5,200,000, decidedly stronger than 1:8,000,000, somewhat

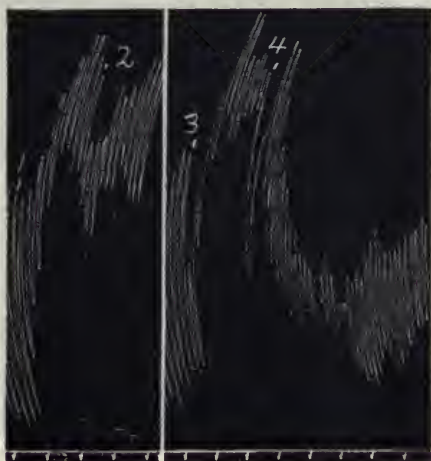


FIG. 10. INTESTINE TRACINGS. BLOODS FROM CAT 285

At 1 and 3 Ringer was replaced by indifferent (jugular) blood and this at 2 by the second adrenal specimen (collected before injection of nicotine); at 4 by the fourth adrenal specimen (collected one minute after injection of nicotine). All the bloods were diluted with three volumes Ringer. (Reduced to one-half.)

stronger than 1:6,600,000. It was finally assayed at 1:6,500,000 corresponding to an output of 0.0007 mgm. per minute for the cat, or 0.00016 mgm. per kilogram per minute.

Collection of the third specimen was begun nominally thirty seconds after the nicotine injection, but allowing for the filling of the dead space in the cava and cannula, the first blood included in the specimen must have left the adrenals not later than fifteen seconds after the injection. The third specimen produced an

inhibitory effect on the intestine segment decidedly greater than that produced by the fourth specimen (fig. 11, observations 28 and 26), although the blood flow was nearly three times as great in the case of the third specimen.

The assay proved that the third specimen had an epinephrin concentration much greater than 1:1,000,000, greater than 1:330,000, less than 1:220,000 (intestine tracings not repro-

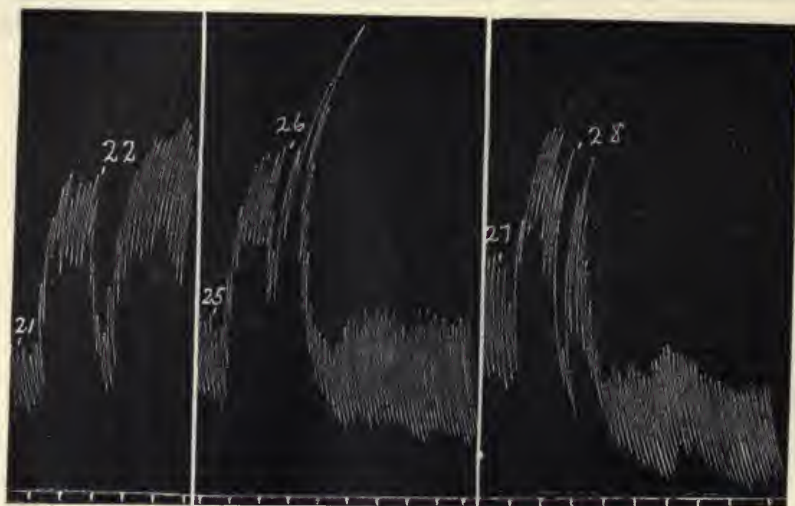


FIG. 11. INTESTINE TRACINGS. BLOODS FROM CAT 285

At 21, 25 and 27 Ringer was replaced by venous blood collected after injection of nicotine; and this at 22 by the seventh adrenal specimen (collected twenty-two minutes after injection of nicotine); at 26 by the fourth adrenal specimen (collected one minute after injection of nicotine); at 28 by the third adrenal specimen (collected immediately after injection of nicotine). All the bloods were diluted with three volumes Ringer. (Reduced to one-half.)

duced, confirmed by uterus, figure 12, observations 76 and 77). It was taken at 1:275,000, corresponding to an output of 0.01 mgm. per minute for the cat, or 0.0023 mgm. per kilogram per minute, fourteen times the initial rate of output.

The average output for the period of collection of the fourth specimen was not nearly one-third of that during the collection of the third specimen. No doubt towards the end of the col-

lection of the fourth specimen it was much less, since the output for the fifth specimen, immediately succeeding the fourth, was no more than half the initial output (0.00035 mgm. per minute for the cat, or 0.0008 mgm. per kilogram per minute).

The seventh specimen, collected twenty-two and one-half minutes after injection of nicotine, when the blood pressure had again risen considerably caused a much smaller inhibition of the intestine segment than either the third or the fourth specimens



FIG. 12. UTERUS TRACINGS. BLOODS FROM CAT 285

At 70 Ringer was replaced by jugular blood to which was added adrenalin to make a concentration of 1:6,600,000; at 71 by the second adrenal specimen (collected before injection of nicotine); at 72 by the seventh adrenal specimen (collected twenty-two minutes after injection of nicotine); at 74 by jugular blood to which was added adrenalin to make a concentration of 1:6,600,000 and also sufficient nicotine to make 0.01 mgm. per cubic centimeter of blood; at 76 by the third adrenal specimen; at 77 by jugular blood to which was added adrenalin to make a concentration of 1:220,000. All the bloods were diluted with three volumes Ringer (the adrenalin bloods after adding the adrenalin). (Reduced to one-half.)

(fig. 11), and a somewhat smaller effect than the second specimen (intestine tracings not reproduced, but confirmed by uterus tracings, figure 12, observations 71 and 72, and by other uterus observations). It was assayed at 1:7,000,000 epinephrin, equivalent to an output of 0.00045 mgm. per minute for the cat, or 0.0001 mgm. per kilogram per minute. Even at this time the original output before nicotine had not yet been regained. The

proportion of serum in the blood was 56.5 per cent as determined by the electrical conductivity method. The hematocrite gave 46 per cent after fifteen minutes rotation; 49.5 per cent after seven minutes more and 52 per cent after a further twelve minutes. With 56 per cent of serum the concentration of epinephrin in the third specimen must have been 1:150,000, a concentration much greater than the normal maximum.

In the next experiment (cat 286) the dose of nicotine was again reduced (to 0.25 mgm. per kilogram) but the depressant effect upon the epinephrin output was as prominent and the preliminary stimulating effect as fleeting as in any of the experiments with larger doses. The depressant effect, indeed, came out more sharply in specimens of adrenal blood collected soon after the nicotine injection than with the larger doses, doubtless because the smaller increase in the output produced in the first stage did not mask the depression in the succeeding specimens by overlapping.

Condensed protocol. Cat 286, male, weight, 4.0 kgm.

Anesthetized with ether. Obtained indifferent blood from jugular. Made cava pocket. Collected adrenal blood.

11.45 a.m. First specimen, 2.5 grams in 30 seconds (5 grams per minute). Second specimen, 7.1 grams in 90 seconds (4.7 grams per minute). Blood pressure during collection of second specimen was 150 mm. mercury (fig. 13, observation 2).

11.48 a.m. Injected 1 mgm. nicotine intravenously (fig. 13, observation 4).

11.48½ a.m. Third adrenal specimen, 1.7 grams in 30 seconds (3.4 grams per minute).

11.49 a.m. Fourth adrenal specimen, 4.15 grams in 90 seconds (3.1 grams per minute).

11.50½ a.m. Fifth adrenal specimen, 5.2 grams in 90 seconds (3.4 grams per minute).

Blood pressure at the beginning of the collection of the third specimen was 130 mm. mercury; (fig. 13. observation 5) at the beginning of the collection of the fourth specimen 108 mm. mercury (observation 6); at the end of the collection of the fifth, 122 mm. mercury, (observation

7). The respiration was good throughout the experiment; artificial respiration was not needed.

12.03 p.m. Sixth adrenal specimen, 2.6 grams in 30 seconds (5.2 grams per minute).

12.03½ p.m. Seventh adrenal specimen, 9.15 grams in 120 seconds (4.6 grams per minute).

12.05½ p.m. Eighth adrenal specimen, 6.5 grams in 90 seconds (4.3 grams per minute).

Blood pressure at the beginning of the collection of the sixth specimen was 146 mm. mercury; (fig. 13, observation 9); at the beginning of

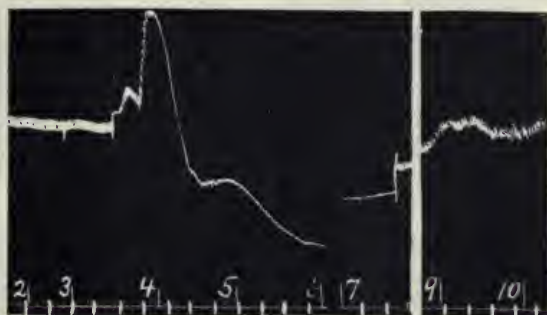


FIG. 13. BLOOD PRESSURE TRACING. CAT 286

2, Blood pressure during collection of second adrenal specimen; 3, end of collection of second specimen; 4, end of injection of 1 mgm. nicotine; 5, beginning of collection of third adrenal specimen; 6, beginning of collection of fourth adrenal specimen; 7, end of collection of fifth adrenal specimen; 9, beginning of collection of sixth adrenal specimen; 10, beginning of collection of seventh adrenal specimen. Zero line corresponds with time trace and is moved up 40 mm. and the figure then reduced to two-thirds.

collection of the seventh specimen 148 mm. mercury (observation 10); and during the collection of the eighth specimen, 148 mm. mercury.

Obtained another specimen of venous blood. Combined weight of adrenals 0.372 gram.

Figure 13 indicates the points on the blood pressure curve at which the adrenal blood specimens were collected. Figure 14 shows that the third adrenal specimen, collected half a minute, or allowing for the dead space, not much more than fifteen seconds, after the nicotine injection, had a much greater concen-

tration of epinephrin than the fourth specimen, collected immediately thereafter, which indeed produced little if any inhibition of the intestine segment. Since the blood flow for the fourth was practically the same as for the third, even a little less, this of itself indicates quite clearly that the output during collection of the third specimen must have been greater than during collection of the fourth. The eighth specimen (collected seventeen and one-half minutes after injection of nicotine) was also decidedly stronger than the fourth, although much weaker than the third.

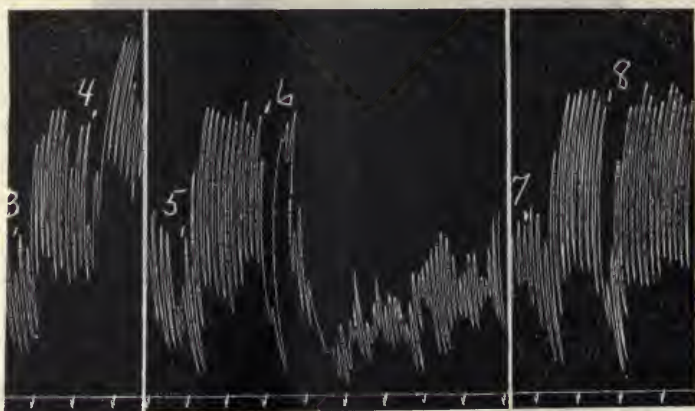


FIG. 14. INTESTINE TRACINGS. BLOODS FROM CAT 286

At 3, 5 and 7 Ringer was replaced by jugular blood and this at 4 by the fourth adrenal specimen (collected one minute after injection of nicotine); at 6 by the third adrenal specimen (collected immediately after injection of nicotine); at 8 by the eighth adrenal specimen (collected seventeen minutes after nicotine injection). All the bloods were diluted with three volumes Ringer. (Reduced to two-thirds.)

In other observations, e.g., observations 40 and 42 in figure 15, it was demonstrated that neither the fourth nor the fifth adrenal blood specimens caused any inhibition of the intestine segment, whereas the eighth specimen always gave a fair inhibition (fig. 15, observation 46). The eighth specimen did not differ much in concentration from the second specimen, collected before injection of nicotine (fig. 16), but was found by the assay

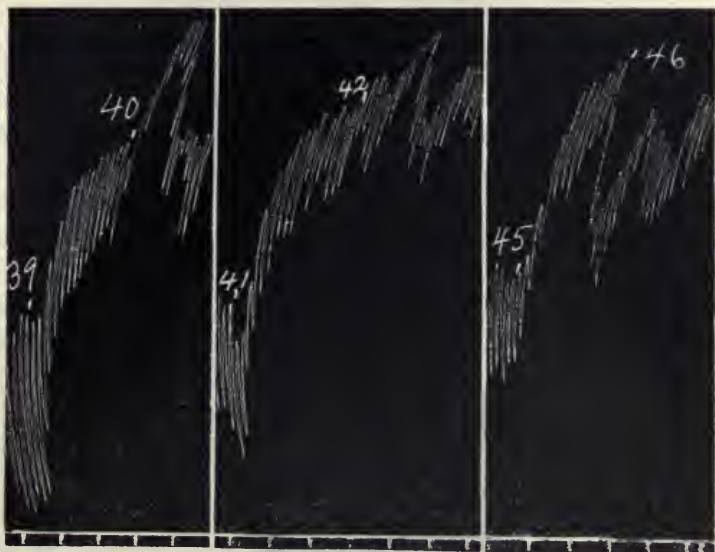


FIG. 15. INTESTINE TRACINGS. BLOODS FROM CAT 286

At 39, 41 and 45 Ringer was replaced by venous blood collected after injection of nicotine; and this at 40 by the fourth adrenal specimen (collected one minute after injection of nicotine); at 42 by the fifth adrenal specimen (collected two and one-half minutes after injection of nicotine); at 46 by the eighth adrenal specimen (collected seventeen minutes after injection of nicotine). All the bloods were diluted with three volumes Ringer. (Reduced to two-thirds.)

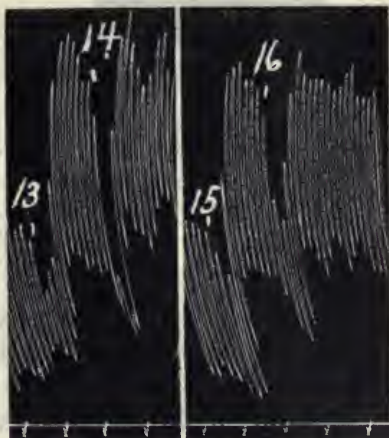


FIG. 16. INTESTINE TRACINGS. BLOODS FROM CAT 286

At 13 and 15 Ringer was replaced by jugular blood, and this at 14 by the eighth adrenal specimen (collected seventeen minutes after injection of nicotine); at 16 by the second adrenal specimen (collected before injection of nicotine). The bloods were diluted with three volumes Ringer. (Reduced to two-thirds.)

to be slightly weaker. It was confirmed by observations on the uterus that the fourth specimen gave practically no effect and certainly no greater effect than indifferent (jugular) blood, while the third specimen caused a very great increase of tone. Indifferent blood made up with adrenalin to a concentration of 1:7,000,000 produced a far greater reaction than the fourth specimen, although far less than the third (fig. 17).

The detailed assay showed that the second specimen was much stronger than 1:10,600,000 adrenalin, stronger than 1:8,000,000,

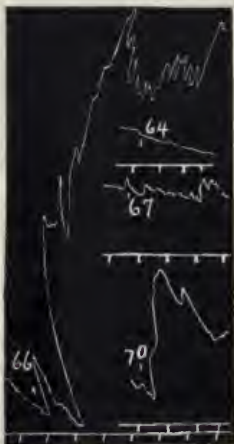


FIG. 17. UTERUS TRACINGS. BLOODS FROM CAT 286

At 64 and 67 Ringer was replaced by the fourth adrenal specimen (collected one minute after injection of nicotine); at 66 by the third adrenal specimen (collected immediately after injection of nicotine); at 70 by jugular blood to which was added adrenalin to make a concentration of 1:7,000,000. All the bloods were diluted with three volumes Ringer (the adrenalin bloods after adding the adrenalin. (Reduced to one-half.)

weaker than 1:5,300,000, and approximately equal to 1:6,500,000, equivalent to an output of epinephrin of 0.00072 mgm. per minute for the cat, or 0.00018 mgm. per kilogram per minute.

The third specimen was found to be much stronger than 1:1,300,000, somewhat stronger than 1:530,000, but decidedly weaker than 1:330,000. It was assayed at 1:500,000, corresponding to an epinephrin output of 0.0068 mgm. per minute for

the cat, or 0.0017 mgm. per kilogram per minute, more than nine times the output before the injection of nicotine. The percentage of serum in the blood was 67, as determined by the electrical method; by the hematocrite 57 with fifteen minutes, 60 with twenty-two minutes, and 62 with thirty minutes rotation. The concentration of epinephrin in the serum of the third specimen would, therefore, be about 1:330,000.

As already stated, the fourth specimen, collected immediately after the third, and the fifth specimen, collected immediately after the fourth, did not contain an amount of epinephrin detectable by the segments worked with, so that if any epinephrin at all was present in these specimens the output during their collection must have been much less than the original output. A period of marked depression lasting for several minutes must, therefore, have succeeded the intense brief stimulation during collection of the third specimen. It is practically certain that this period of stimulation was entirely over before the end of collection of that specimen, else the fourth specimen would necessarily have been overlapped and must have contained epinephrin in detectable amount. It is, accordingly improbable that the stimulation in this experiment lasted more than a half minute, if so long.

The eighth specimen was shown to be weaker than 1:5,700,000 adrenalin, stronger than 1:6,660,000, somewhat stronger than 1:6,000,000. It was taken at 1:5,850,000, corresponding to an output of 0.00073 mgm. per minute for the cat, or 0.00018 mgm. per kilogram per minute, the same as in the second specimen, taken before nicotine injection. Recovery from the depressant effect was therefore complete at this time, seventeen and one-half minutes after the administration of the drug.

The seventh specimen, collected just before the eighth, had a smaller concentration, but in the absence of an exact assay of this specimen, it is not possible to know whether the output was smaller, as the flow for the seventh was somewhat larger than for the eighth.

The next experiment (cat 298) was performed with the object of assaying by the colorimetric method of Folin, Cannon and

Denis (4) the adrenal blood specimen taken immediately after the injection of nicotine. It was argued that with concentrations of epinephrin as great as had been observed previously during the brief preliminary period of stimulation by the drug a definite colorimetric reaction ought to be obtained. This would, of course, constitute the best possible corroboration of the results deduced from the assays on the segments. The colorimetric method is not delicate enough to be used with the epinephrin concentrations ordinarily present in adrenal blood.

Condensed protocol. Cat 298, female, weight 3.35 kgm.

Anesthetized with ether. Obtained indifferent blood from jugular. Made cava pocket. Collected adrenal blood.

11.03 a.m. First specimen, 1.7 grams in 45 seconds (2.2 grams per minute).

11.03 $\frac{3}{4}$ a.m. Second specimen, 5.35 grams in 180 seconds (1.8 grams per minute). Blood pressure during collection of second specimen was 80 mm. mercury (fig. 18, observation 4).

11.10 $\frac{1}{2}$ a.m. Injected 2 mgm. nicotine intravenously (fig. 18, observation 5).

11.11 a.m. Third adrenal specimen, 2.4 grams in 60 seconds (2.4 grams per minute).

11.12 a.m. Fourth adrenal specimen, 5.55 grams in 360 seconds (0.92 gram per minute).

Blood pressure at the beginning of the collection of the third specimen was 110 mm. mercury (fig. 18, observation 6); at the beginning of the collection of the fourth specimen 60 mm. mercury (observation 7).

11.23 a.m. Obtained another specimen of indifferent blood from jugular (nicotine blood).

11.28 $\frac{1}{2}$ a.m. Fifth adrenal specimen, 1.7 grams in 45 seconds (2.2 grams per minute).

11.29 $\frac{1}{4}$ a.m. Sixth adrenal specimen, 5.6 grams in 180 seconds (1.9 grams per minute).

Blood pressure at the beginning of the collection of the fifth specimen was 76 mm. mercury; at the beginning of the collection of the sixth specimen 78 mm. mercury (fig. 18, observation 10).

Obtained another specimen of venous blood. Combined weight of adrenals 0.418 gram.

The expectation was realized. A dose of nicotine was chosen which seemed likely to be large enough to give a strong effect, but yet not so large as to cut short the stimulation almost instantaneously by the succeeding paralysis. As in a cat it was not possible to obtain enough blood in a half minute collection to permit a complete assay on the segments as well as the colorimetric assay, it was decided to limit the segment assay for the third specimen to determining a concentration of adrenalin which was clearly less than that of the specimen. The other specimens

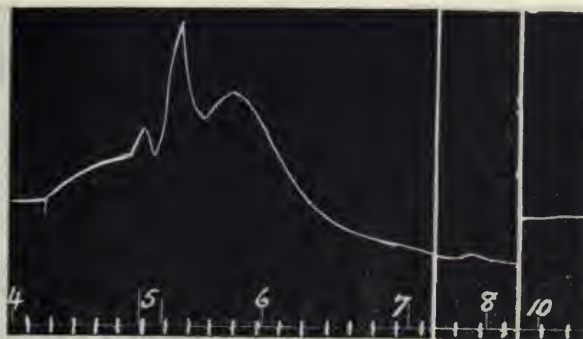


FIG. 18. BLOOD PRESSURE TRACING. CAT 298

4, End of collection of second adrenal specimen; 5, injection of nicotine; 6, beginning of collection of third adrenal specimen; 7, beginning of collection of fourth adrenal specimen; 8, end of collection of fourth adrenal specimen; 10, beginning of collection of sixth adrenal specimen. Zero line corresponds with time trace and is moved up 15 mm. and the figure then reduced to two-thirds.

were carefully assayed as usual by the segment reactions. Figure 18 shows the points on the blood pressure tracing at which the adrenal specimens were collected.

The second adrenal blood specimen, collected before injection of nicotine, was found to be decidedly stronger than 1:4,000,000 adrenalin, much weaker than 1:1,300,000, weaker than 1:2-660,000 (confirmed by several observations). It was taken at 1:3,000,000, corresponding to an output of epinephrin of 0.0006 mgm. per minute for the cat, or 0.00018 mgm. per kilogram per minute.

The third adrenal specimen, the collection of which was begun half a minute after the nicotine injection (allowing for the dead space within fifteen to twenty seconds after the injection), gave an enormously greater inhibition with the intestine segment than any of the other specimens (fig. 19, observation 27). It was shown by the uterus segment that the blood was certainly not weaker than 1:330,000 epinephrin. The serum was approximately assayed by the colorimetric method at 1: 300,000. The blood was very rich in serum (over 80 per cent). Blood with a

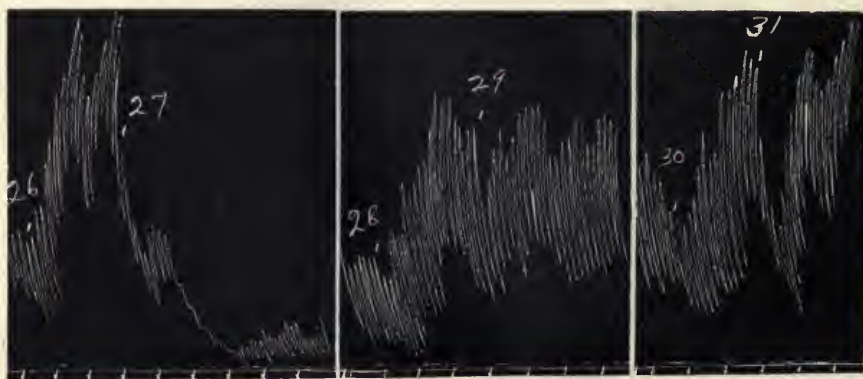


FIG. 19. INTESTINE TRACINGS. BLOODS FROM CAT 298

At 26, 28 and 30 Ringer was replaced by jugular blood obtained after nicotine injection; and this at 27 by the third adrenal specimen (collected immediately after the nicotine injection); at 29 by the fourth adrenal specimen (collected one and one-half minutes after nicotine injection); at 31 by the sixth adrenal specimen (collected nineteen minutes after nicotine injection). All the bloods were diluted with three volumes Ringer. (Reduced to four-sevenths.)

concentration of 1:330,000 would correspond to serum with a concentration of 1:265,000. The epinephrin output, taking the concentration of the third specimen at 1:330,000 would be 0.0073 mgm. per minute for the cat, or 0.0022 mgm. per kilogram per minute, more than twelve times the initial output before nicotine.

The fourth adrenal specimen, taken immediately after the third, gave hardly any reaction with the intestine segment (fig. 19, observation 29, no inhibition at all in another observation),

although the blood flow during its collection was not much more than one-third of that for the third specimen. It was shown that the output at the time the fourth specimen was taken (beginning one and one-half minutes after the nicotine injection, or about one and one-quarter minutes allowing for lag due to the dead space) must have been much less than 0.00006 mgm. per kilogram per minute, since its concentration was much less than 1:4,000,000 epinephrin, if any at all was present. In other words, the rate of output at the time of collection of the fourth specimen was much less than one-fortieth of the rate for the third. This indicates clearly that the period of excitation was completely over before the end of collection of the third specimen, i.e. (allowing for the dead space), at most seventy-five seconds after the administration of nicotine. As it is quite improbable that the beginning of the fourth specimen coincided exactly with the end of the period of excitation, the duration of the latter may be assumed to have been less than a minute and a quarter. If only half of the third specimen passed through the adrenals during the period of excitation the concentration of epinephrin in this portion of the blood must have been twice the concentration calculated on the whole specimen, and the increase in the rate of output as compared with the output before nicotine double the calculated increase for the whole specimen.

The sixth adrenal specimen, collected about nineteen minutes after the injection of nicotine, gave a much greater reaction with the intestine segment than the fourth (fig. 19, observation 31), although the blood flow was twice as great. It was assayed at 1:4,000,000 epinephrin, corresponding to an output of 0.00047 mgm. per minute for the cat, or 0.00014 mgm. per kilogram per minute. In the eleven minutes between the end of collection of the fourth and the beginning of collection of the sixth specimen the output had accordingly recovered considerably, although it was not yet quite equal to the original output before nicotine.

Two experiments were made with much smaller doses than in any of the preceding experiments, in order to see whether the smaller doses might not bring out more clearly the stimulation

effect in comparison with the depression of the output. One of the animals received 0.1 mgm. per kilogram of bodyweight and the other 0.08 mgm. per kilogram. As in making the cava pocket the abdominal aorta is tied near the bifurcation, a portion of the animal is excluded from the circulation. This has not been taken into account in calculating the dosage, nor do we know that any allowance should be made for it, as the structures mainly acted upon by nicotine and the other drugs studied are not affected by this ligation.

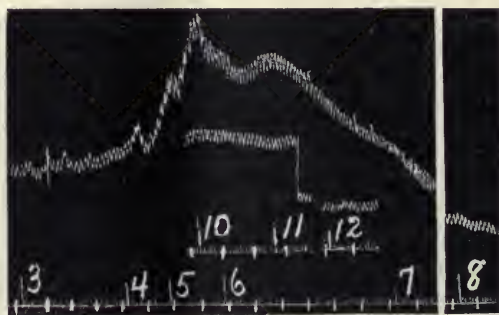


FIG. 19A. BLOOD PRESSURE TRACING. CAT 311

3, End of collection of second adrenal specimen; 4 to 5 intravenous injection of 0.4 mgm. nicotine; 6, beginning of collection of third adrenal specimen; 7, beginning of collection of fourth specimen; 8, beginning of collection of fifth specimen; 10, beginning of collection of sixth specimen; 11, beginning of collection of seventh specimen; 12, end of collection of seventh specimen. Zero line corresponds with time trace and is moved up 18 mm. and the figure then reduced to two-thirds.

With these small doses just as with the larger doses, the depressant action was the predominant and the most easily demonstrated effect. Indeed, relatively to the excitation the depression was more manifest than with the larger doses. The condensed protocol of one of the experiments is given as an example.

Condensed protocol. Cat 311, male, weight, 3.89 kgm.

Anesthetized with ether. Obtained indifferent blood from jugular. Made cava pocket. Collected adrenal blood.

- 10.27 a.m. First specimen, 2.9 grams in 30 seconds (5.8 grams per minute).
- 10.27½ a.m. Second specimen, 7.1 grams in 90 seconds (4.7 grams per minute). Blood pressure at end of collection of second adrenal specimen was 90 mm. of mercury (fig. 19a, observation 3).
- 10.34 a.m. Injected 0.4 mgm. nicotine intravenously (fig. 19a, observations 4 and 5).
- 10.34½ a.m. Third adrenal specimen, 6.7 grams in 60 seconds (6.7 grams per minute).
- 10.35½ a.m. Fourth adrenal specimen, 5.15 grams in 90 seconds (3.4 grams per minute).
- 10.37 a.m. Fifth adrenal specimen, 5.55 grams in 120 seconds (2.2 grams per minute).

Blood pressure at beginning of collection of third adrenal specimen was 136 mm. of mercury (fig. 19a, observation 6), at beginning of collection of fourth adrenal specimen 97 mm. (observation 7), at beginning of collection of fifth adrenal specimen 70 mm. (observation 8).

- 10.48½ a.m. Sixth adrenal specimen, 1.75 grams in 30 seconds (3.5 grams per minute).
- 10.49 a.m. Seventh adrenal specimen, 7.45 grams in 180 seconds (2.5 grams per minute).

Blood pressure at beginning of collection of sixth specimen was 84 mm. of mercury (fig. 19a, observation 10), at beginning of collection of seventh specimen 76 mm. (observation 11), at the end of collection of seventh specimen 54 mm. (observation 12). Obtained another specimen of venous blood. Combined weight of adrenals 0.668 gram.

The second specimen, (before nicotine) corresponded to an output of epinephrin of 0.0006 mgm. per minute for the cat, or 0.00015 mgm. per kilogram per minute. The third specimen (collected twenty seconds, or allowing for the dead space, ten to fifteen seconds after the end of the nicotine injection) corresponded to an output of 0.00065 mgm. per minute for the cat, or 0.00016 mgm. per kilogram per minute, practically the same as the original output. That some augmentation was present in the early part of the period of collection of this specimen was indicated clearly by the fact that the fourth specimen, taken immediately after the third, corresponded to an output of only

0.00025 mgm. per minute for the cat, or 0.00006 mgm. per kilogram per minute, less than half the rate for the third specimen. Since it is very improbable that the depressant action began precisely with the collection of the fourth specimen, some augmentation must have been present in the early part of the third specimen, to make up for the depression during the latter part.

The fifth specimen, taken immediately after the fourth (beginning three minutes after the nicotine injection) already showed some recovery in the epinephrin output as compared with the fourth specimen (to 0.00044 mgm. per minute for the cat, or 0.00011 mgm. per kilogram per minute). When the seventh specimen was obtained (fifteen minutes after the nicotine injection) the original output had been regained (0.0006 mgm. per minute for the cat, or 0.00015 mgm. per kilogram per minute), although the blood pressure was considerably lower than at the time of collection of the second specimen. It must be remembered that in an experiment of this kind, the complete restoration of conductivity in the vasomotor efferent path, does not necessarily carry with it restoration of the blood pressure to the original level. The animal has been losing some blood. It has been kept under an anesthetic after an operation, and the blood pressure under these conditions may be gradually falling. The removal of the nicotine block will not then of course restore the pressure completely, although the epinephrin output may come back to the initial amount. The more prompt recovery with the smaller doses is what might be expected.

EXPERIMENTS WITH HYPODERMIC INJECTION OF NICOTINE AND ASSAY OF ADRENAL BLOOD ON RABBIT SEGMENTS

Two experiments were made with hypodermic injection of nicotine, in order to see whether with a more gradual action of the poison, the preliminary period of increased output might not be prolonged relatively to the succeeding period of depression. This was not found to be the case. On the contrary, the depressant effect of nicotine upon the epinephrin output asserted itself even more obviously than with intravenous injection, as the outstanding action of the drug upon the secretion.

Condensed protocol. Cat 303, male, weight, 4.82 kgm.

Anesthetized with ether. Obtained indifferent blood from jugular. Made cava pocket. Collected adrenal blood.

10.27 a.m. First specimen, 3.5 grams in 30 seconds (7 grams per minute).

10.27½ a.m. Second specimen, 9.05 grams in 90 seconds (6 grams per minute).

Blood pressure during collection of second specimen was 136 mm. mercury (fig. 20, observation 4).

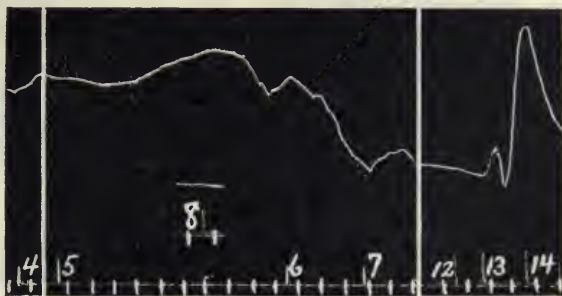


FIG. 20. BLOOD PRESSURE TRACING. CAT 303

4, End of collection of second adrenal specimen; 5, injected 5 mgm. nicotine subcutaneously; 6, beginning of collection of third adrenal specimen; 7, beginning of collection of fourth adrenal specimen; 8, beginning of collection of fifth adrenal specimen; 12, end of collection of seventh adrenal specimen; 13, injected 1 mgm. nicotine intravenously; 14, beginning of collection of eighth adrenal specimen. Zero line corresponds with time trace and is moved up 20 mm. and the figure then reduced to two-thirds.

10.34 a.m. Injected 5 mgm. nicotine subcutaneously (fig. 20, observation 5).

10.36 a.m. Third adrenal specimen, 2.55 grams in 30 seconds (5.1 grams per minute).

10.36½ a.m. Fourth adrenal specimen, 3.6 grams in 90 seconds (2.4 grams per minute).

10.38 a.m. Fifth adrenal specimen, 5.25 grams in 150 seconds (2.1 grams per minute).

Blood pressure at beginning of collection of third specimen was 120 mm. mercury (fig. 20, observation 6); at the beginning of collection of the fourth specimen 83 mm. mercury (fig. 20, observation 7); at the

beginning of collection of the fifth specimen 60 mm. mercury (observation 8).

10.51 a.m. Sixth adrenal specimen, 2.4 grams in 30 seconds (4.8 grams per minute).

10.51½ a.m. Seventh adrenal specimen, 8.3 grams in 120 seconds (4.2 grams per minute).

Blood pressure 88 mm. mercury (fig. 20, observation 12).

10.53¾ a.m. Injected 1 mgm. nicotine intravenously (fig. 20, observation 13).

10.54 a.m. Eighth adrenal specimen, 3.95 grams in 60 seconds (3.95 grams per minute).

10.55 a.m. Ninth adrenal specimen, 2.2 grams in 120 seconds (1.1 grams per minute).

At the beginning of the collection of the eighth specimen the blood pressure was up to 144 mm. mercury (fig. 20, observation 14); then it fell to 45 mm. mercury. Obtained another specimen of venous blood. Combined weight of adrenals 0.66 gram.

In the first of these experiments (cat 303), with a relatively large dose of nicotine (5 mgm.) the stimulation effect was missed altogether even in the adrenal blood specimen collected two minutes (about one and three-quarter minutes, allowing for the dead space) from the beginning of the nicotine injection. Further experiments showed, however, that a period of increased output would unquestionably have been detected in this cat had the first adrenal sample after nicotine been taken earlier. Figure 20 indicates the positions of the adrenal blood specimens on the blood pressure tracing.

The second specimen (taken before injection of the drug) was found to be stronger than 1:6,660,000, and weaker than 1:5,300,000 adrenalin (confirmed for each limit by 2 separate pairs of observations made at different times with the same intestine segment). Taking the second specimen at 1:6,000,000, we get 0.001 mgm. of epinephrin per minute for the cat, or 0.0002 mgm. per kilogram per minute.

The third specimen, collected two minutes after the hypodermic nicotine injection (one and three-quarter minutes, allowing for the dead space) was found to have a somewhat greater concentration of epinephrin than the second. It was distinctly

weaker than 1:4,000,000, stronger than 1:6,660,000, and was assayed at 1:5,200,000, equivalent to an output of 0.001 mgm. per minute for the animal, or 0.0002 mgm. per kilogram per minute, the same as for the second specimen.

Subsequent experience enables us to interpret these results. They do not mean that the relatively large dose of nicotine administered hypodermically produced no effect upon the epinephrin output, but that a brief period of increased output had intervened, somewhere between the injection and the beginning of collection of the third specimen. This increased output lapped over to some extent into this sample, maintaining approximately the same output as in the second specimen in the face of the rapidly developing depression. It is not known whether the depression had reached its maximum before collection of the seventh specimen (seventeen and one-half minutes after the nicotine injection) or was still developing. Probably the maximum depression occurred somewhere between the fourth and seventh specimens for the blood pressure had recovered considerably when the latter was collected. The seventh adrenal specimen had a much smaller concentration of epinephrin than the fourth, but the blood flow was nearly twice as great. The seventh was somewhat weaker than the second. It was weaker than 1:6,660,000, somewhat stronger than 1:8,000,000, and was taken at 1:7,200,000, corresponding to an output of 0.0006 mgm. per minute for the cat, or 0.00012 mgm. per kilogram per minute. At this time then the output had not yet reached its original value before nicotine.

At this point a dose of nicotine (1 mgm.) was injected intravenously in order to ascertain whether the transient preliminary stimulating effect could be demonstrated in this animal when an adrenal blood sample was collected immediately after the injection. The result was positive, as in all the experiments with intravenous injection. The eighth specimen was collected fifteen seconds, or allowing for the dead space, only a few seconds after the nicotine injection. The pocket had been clipped off before the collection of the sixth specimen, and the adrenal blood was continuously collected till the end of the ninth specimen.

The nicotine was injected while collection was going on, and collection of the eighth specimen was begun at the moment when the blood pressure began to rise after the nicotine injection. The fact that an excellent rise of pressure was produced by the nicotine, although no epinephrin was entering the circulation, or had entered it for three minutes, is of interest in connection with Gley's theory, already criticized in the introduction to this paper, of the mode of action of nicotine in increasing the blood pressure.

The eighth adrenal blood specimen was proved by the assay to be weaker than 1:1,300,000, stronger than 1:2,700,000, and not far from 1:2,000,000 epinephrin, equivalent to an output of 0.002 mgm. per minute for the cat, or 0.0004 mgm. per kilogram per minute, more than three times the output just before the intravenous injection and twice the output at the beginning of the experiment.

In the other experiment with hypodermic injection of the drug (cat 305) a smaller dose was employed and the adrenal blood was collected at a shorter interval after the injection.

Condensed protocol. Cat 305, male, weight, 3.41 kgm.

Anesthetized with ether. Obtained indifferent blood from jugular. Made cava pocket. Collected adrenal blood.

10.53½ a.m. First specimen, 1.6 grams in 30 seconds (3.2 grams per minute).

10.54 a.m. Second specimen, 6.8 grams in 120 seconds (3.4 grams per minute).

Blood pressure during collection of the second specimen was 140 mm. mercury (fig. 21, observation 4).

11.00 a.m. Injected 2 mgm. nicotine subcutaneously (fig. 21, observation 5).

11.01 a.m. Third adrenal specimen, 4.25 grams in 60 seconds (4.25 grams per minute).

11.02 a.m. Fourth adrenal specimen, 4 grams in 90 seconds (2.7 grams per minute).

11.03½ a.m. Fifth adrenal specimen, 4.9 grams in 150 seconds (1.96 grams per minute).

Blood pressure at the beginning of the collection of the third specimen was 172 mm. mercury (fig. 21, observation 6); at the beginning of the collection of the fourth specimen, 154 mm. mercury (observation 7); and at the beginning of the collection of the fifth specimen 108 mm. mercury (observation 8).

11.18 a.m. Sixth adrenal specimen.

11.18½ a.m. Seventh adrenal specimen, 3.6 grams in 180 seconds (1.2 grams per minute).

Blood pressure at the end of collection of the seventh specimen was 76 mm. mercury (fig. 21, observation 12).

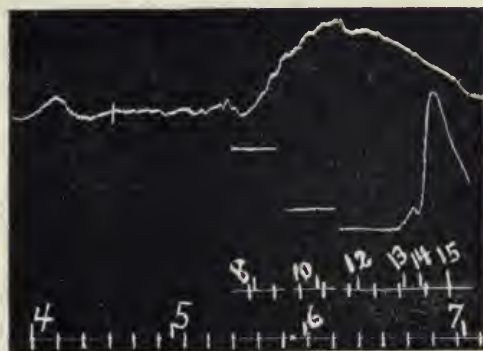


FIG. 21. BLOOD PRESSURE TRACING. CAT 305

4, End of collection of second adrenal specimen; 5, subcutaneous injection of 2 mgm. nicotine; 6, beginning of collection of third adrenal specimen; 7, beginning of collection of fourth adrenal specimen; 8, beginning of collection of fifth adrenal specimen; 10, beginning of collection of sixth adrenal specimen; 12, end of collection of seventh adrenal specimen; 13 to 14, intravenous injection of 1 mgm. nicotine; 15, beginning of collection of eighth adrenal specimen. Zero line corresponds with time trace and is moved up 26 mm. and the figure then reduced to two-thirds.

11.22 a.m. Injected 1 mgm. nicotine intravenously (fig. 21, observations 13 to 14).

11.22¼ a.m. Eighth adrenal specimen, 1.5 grams in 60 seconds (1.5 grams per minute).

The collection of the eighth specimen was begun at the height of the nicotine rise when the blood pressure had reached a maximum of 130 mm. mercury (fig. 21, observation 15). Allowing for the dead space, the

first drops of the eighth specimen must have left the adrenals about the time the rise was beginning.

Obtained another specimen of venous blood. Combined weight of adrenals 0.312 gram.

The points on the blood pressure curve at which the adrenal blood specimens were procured are indicated in figure 21.

The second adrenal specimen, collected before hypodermic injection of nicotine, was shown by the assay to be much weaker than 1:4,300,000 adrenalin, weaker than 1:5,700,000, stronger than 1:7,000,000. It was confirmed by other observations that the concentration of the specimen was between 1:7,000,000, and 1:5,700,000, and nearer the latter. It was taken at 1:6,000,000, corresponding to an output of 0.00057 mgm. per minute for the cat, or 0.00017 mgm. per kilogram per minute.

The third specimen was procured about a minute after the injection (about twenty seconds after the beginning of the rise of pressure, or only five to ten seconds after the beginning of the rise when the filling of the dead space is corrected for). It was found to be stronger than 1:4,300,000 adrenalin, and decidedly weaker than 1:3,000,000. It was finally assayed at 1:3,500,000 equivalent to an output of 0.0012 mgm. per minute for the cat, or 0.00035 mgm. per kilogram per minute, about double the output before the nicotine injection.

The fourth specimen, collected immediately after the third, beginning about two minutes after the administration of nicotine and little more than a minute after the commencement of the rise of pressure, gave practically the same reaction with the intestine segment as the third specimen (confirmed by two pairs of observations). It was proved to be stronger than 1:4,300,000, weaker than 1:3,000,000 adrenalin, and approximately equivalent to 1:3,500,000. As the blood flow for the third specimen was 50 per cent greater than for the fourth, the output was already diminished at this time, amounting to 0.0008 mgm. per minute for the cat, or 0.00023 mgm. per kilogram per minute. As the next adrenal specimen, the fifth, taken immediately after the fourth, caused no inhibition of the intestine segment, although the blood flow was smaller, it is practically certain that a marked depres-

sion of the epinephrin liberation was already in evidence during the latter part of the period of collection of the fourth specimen, the epinephrin content of this specimen being largely accounted for by the overlapping of the period of stimulation into the first part of the fourth specimen. It is not known, of course, but from other experiments it is probable that during the first half minute of the period of collection of the third specimen the output was materially greater than during the second half minute. What is beyond doubt is that about two and one-half minutes after the absorption of the nicotine had reached the point when an effect on the blood pressure was beginning to manifest itself, the depressant action was already so strong that an intestine segment which could easily have detected a concentration of 1:10,000,000 epinephrin (in blood made up to this concentration and then diluted with three volumes of Ringer's solution) and indeed considerably less than this, gave no inhibition whatever with the fifth specimen. The output at this time accordingly could not have been as much as 0.0002 mgm. per minute for the cat, or 0.00006 mgm. per kilogram per minute, i.e., not one-third of the output before nicotine. The segment was not very sensitive, so that it is impossible to know how much below this the output had sunk.

Fourteen and a half minutes later, i.e., eighteen and one-half minutes after the nicotine injection, the depressant action was still so strong that the seventh adrenal blood specimen also caused no inhibition of the intestine segment (fig. 22, observation 69), although the blood flow was slower than for the fifth specimen. Indifferent blood made up with adrenalin to 1:7,000,000, and then diluted to the same extent as the seventh specimen (with three volumes of Ringer's solution) gave a definite inhibition, although with 1:14,000,000 there was no reaction at this time (fig. 22, observations 71 and 73). It was shown that the seventh specimen could not have contained 1:10,000,000 epinephrin. The output at this time accordingly must have been less than 0.00012 mgm. per minute for the cat, or 0.000035 mgm. per kilogram per minute, i.e., less than one-fifth of the output before nicotine. Subcutaneous injection,

then, instead of accentuating the preliminary excitation of nicotine on the epinephrin output at the expense of the depressant effect, brings out the latter more clearly than with intravenous injection, as the predominant action of the drug. It is not known how much longer the depressant effect would have lasted. That it was not over at the end of collection of the seventh specimen is indicated by the fact that the blood pressure had not yet begun to recover.

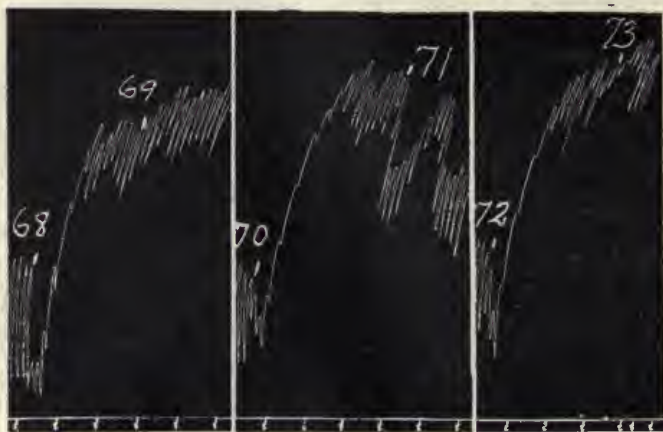


FIG. 22. INTESTINE TRACINGS. BLOODS FROM CAT 305

At 68, 70 and 72 Ringer was replaced by venous blood obtained after injection of nicotine; and this at 69 by the seventh adrenal specimen (collected eighteen minutes after subcutaneous injection of nicotine); at 71, by venous blood to which was added adrenalin to make a concentration of 1:7,000,000; at 73 by venous blood to which was added adrenalin to make a concentration of 1:14,000,000. All the bloods were diluted with three volumes Ringer (the adrenalin bloods after adding the adrenalin). (Reduced to two-thirds.)

Immediately after collection of the seventh specimen, an intravenous injection of nicotine was made and the eighth adrenal specimen collected within fifteen seconds of the beginning of the injection, or allowing for the dead space, almost simultaneously with the completion of the injection, and just as the blood pressure was beginning to rise. The eighth specimen was found to be much stronger than 1:1,400,000, somewhat stronger than

1:700,000, much weaker than 1:430,000 adrenalin (fig. 23). It was taken at 1:600,000, corresponding to an output of 0.0025 mgm. epinephrin per minute for the cat, or 0.0007 mgm. per kilogram per minute. This is four times the initial output before the subcutaneous injection of nicotine. In spite of the depression of the secretory path just before the intravenous injection, the depression was at once changed into excitation, just as in the case of the vasomotor peripheral neurones.

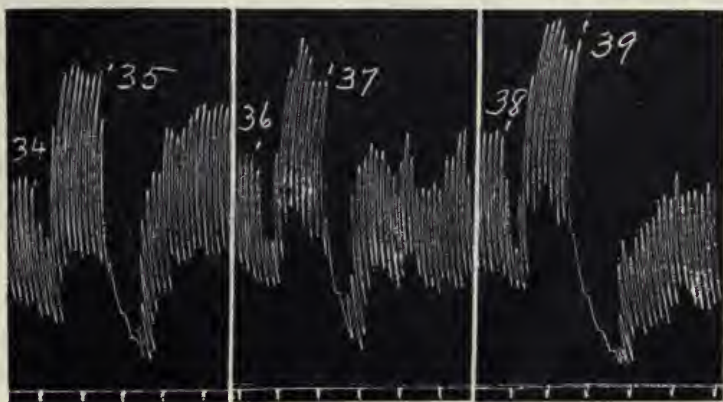


FIG. 23. INTESTINE TRACINGS. BLOODS FROM CAT 305

At 34, 36 and 38 Ringer was replaced by venous blood obtained after injection of nicotine; and this at 35 by venous blood to which was added adrenalin to make a concentration of 1:700,000; at 37 by the eighth adrenal specimen (collected immediately after intravenous injection of nicotine); at 39 by venous blood to which was added adrenalin to make a concentration of 1:430,000. All the bloods were diluted with three volumes Ringer (the adrenalin bloods after adding the adrenalin). (Reduced to two-thirds.)

The percentage of serum in the blood was 54, as determined by the electrical method; (by the hematocrite, 43 with ten minutes, 48.5 with twenty minutes, and 51.5 with thirty-two and one-half minutes rotation). The concentration of epinephrin in the serum of the eighth specimen was, therefore, about 1:320,000.

To sum up: the experiments made by the direct method of assaying the adrenal blood, collected at a known rate, on rabbit segments, show that the action of nicotine, whether administered

intravenously or hypodermically, upon the epinephrin output of the adrenals is essentially a depressant or paralysing action, which comes on early, lasts a relatively long time and only gradually, according to the dose and to other conditions, disappears. The liberation of epinephrin during the period of maximum depression is reduced to a small fraction of the original output, or within the limits of sensitiveness of the test objects, the discharge may cease entirely just as it does when all the secretory fibres are cut. The depressant action is preceded by a transient stimulation during which the rate of output of epinephrin is increased. The duration of the period of excitation probably varies to some extent with the dose, the method of administration and other circumstances, but in general it is measured by half or quarter minutes rather than by minutes, whereas the stage of depression, with the doses employed, may last for many minutes. It was rare to find evidence of the stage of excitation lasting as long as one minute. Because of overlapping of the blood specimens it would require very numerous experiments to fix precise limits for so brief an effect. But in the majority of the experiments the duration of the increased epinephrin output was from half a minute to a minute.

Although it would be difficult to obtain and to assay a sufficient number of adrenal blood samples to make a good curve representing the changes in the rate of epinephrin output throughout an experiment, there is little doubt that such a curve would be roughly parallel to the curve of blood pressure, indicating that the nicotine action on the sympathetic ganglion cells of the efferent vasomotor path is similar to the nicotine action on the sympathetic ganglion cells, or whatever structures may represent them, if there are no sympathetic ganglion cells (6), on the path of the fibres which govern the liberation of epinephrin.

It is obvious from the above results that writers (7), who state that a marked augmentation of the epinephrin discharge in cats is present from three or four to ten or twelve minutes after the intravenous injection of nicotine in doses not very different from the highest doses used by us and that the augmentation may indeed be more pronounced after the longer interval, while making no mention of any depressant effect, must

have employed a faulty method. The period of augmentation must have been over even before they collected their earlier blood specimens after nicotine. The arterial blood pressure and the blood flow in the cava being much reduced, however, they might easily obtain samples of blood after nicotine with a greater epinephrin concentration than the specimen taken before the nicotine injection. The later specimens would be quite likely to have a greater concentration than the earlier specimens after nicotine, because the depressing effect of the drug on the output would probably have been recovered from to some extent when the later specimens were collected. But as already pointed out, the slowing of the circulation would necessarily augment the concentration if no increase occurred in the rate of epinephrin output, or even if a decrease occurred which was proportionally less than the decrease in the rate of blood flow. In the absence of information as to changes in the rate of the blood flow, which cannot be obtained by the catheter method, no conclusions can be drawn as to changes in the rate of output of epinephrin from changes in its concentration, even in the pure adrenal vein blood.

Two or three years ago, being curious to know at first hand what effects nicotine might produce upon the concentration of epinephrin in the cava blood collected in the neighborhood of the adrenals, although aware that information could not be obtained in this way as to changes in the rate of output of epinephrin, we performed several experiments by the catheter method. These are of interest in the present connection, and a condensed protocol of one of them (cat 10) with a small sample of the tracings from the intestine segment (fig. 24) is reproduced. In this experiment, and in some of the others we opened the abdomen, so as to be able to clip the adrenal veins at will, or to leave them free. The results did not differ essentially whether the abdomen was opened or not.

Condensed protocol. Cat 10, female, weight, 2.95 kgm.

Anesthetized with ether. Exposed both femoral veins; opened abdomen and isolated both adrenal veins.

Inserted catheter 40 mm. into right femoral vein and obtained first specimen.

Inserted catheter up to level of adrenals and obtained second specimen. Injected into femoral vein 5 mgm. nicotine, and 3 minutes later inserted catheter up to level of adrenals and obtained third specimen. Artificial respiration begun during the collection of third specimen. Now

clipped off both adrenal veins, and collected through a catheter the fourth specimen (9 minutes after collection of third specimen).

Five minutes after collecting the fourth specimen (i.e., 17 minutes after the nicotine injection) obtained from catheter at the adrenal level the fifth specimen. The blood pressure at this stage was very low. A cava pocket was made and a small sample of adrenal vein blood collected.

A sample of arterial blood was finally obtained.

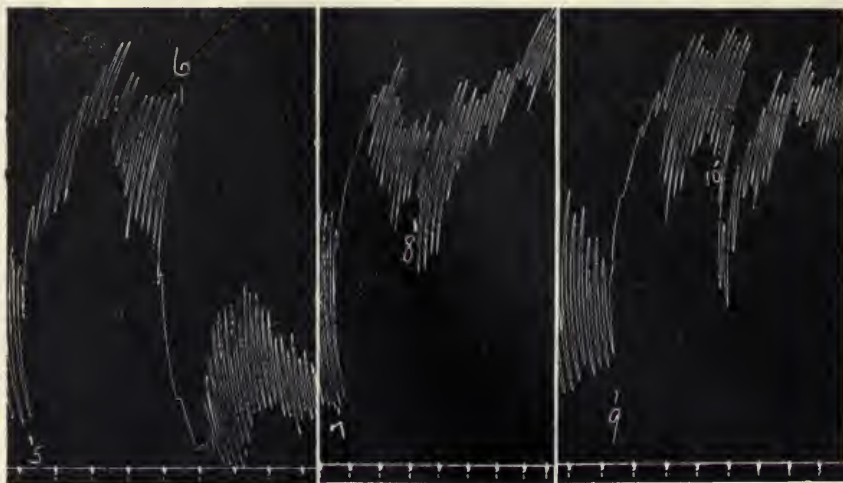


FIG. 24. INTESTINE TRACINGS. BLOODS FROM CAT 10

At 5 Ringer was replaced by catheter blood obtained from the lower cava, and this at 6 by adrenal vein blood; at 7 and 9 Ringer was replaced by catheter blood drawn from the cava at adrenal level after nicotine injection but after clipping off both adrenal veins, and this at 8 by catheter blood obtained from the level of the adrenals (with adrenal veins open) three minutes after injection of nicotine; at 10 by catheter blood obtained from the same level (with adrenal veins open) seventeen minutes after the nicotine injection.) (Reduced to one-half.)

The catheter was rinsed with Ringer's fluid and freshly oiled between collection of the specimens. The collections were made by aspirating slowly with the aid of a syringe.

Cava blood from the adrenal level before nicotine, displacing the lower cava blood, gave slight inhibition of the intestine segment, while cava blood from the adrenal level 3 minutes after the injection caused no effect. To eliminate any action of the nicotine in the blood on the segment, observations were then made in which cava blood from the

adrenal level, obtained with the adrenal veins clipped, was displaced by cava blood from the same level with the adrenal veins open. The specimen taken 3 minutes after injection of nicotine, gave no inhibition of the segment, whereas the specimen obtained 17 minutes after the nicotine injection caused a fair inhibition (fig. 24, observations 8 and 10).

The same result was obtained when indifferent blood drawn with the catheter from the lower part of the cava, was displaced by the 3 minute and the 17 minute specimens, the former gave no reaction, the latter a fair effect. When the 3 minute specimen was displaced by the 17 minute specimen, a good inhibition was produced, confirming the conclusion that the concentration of epinephrin was greater in the latter. No attempt was made to assay the concentration in the 17 minute specimen, as in the absence of data on the rate of blood flow no conclusion could have been arrived at in regard to the effect of nicotine upon the epinephrin output, but it was shown that it was much less than 1:7,000,000, and, of course, very much less than that of pure adrenal vein blood (fig. 24, observation 6). Tests with the rabbit uterus confirmed the intestine observations as to the relative concentration of epinephrin in the various specimens.

EXPERIMENTS WITH AUTO-ASSAY BY BLOOD PRESSURE REACTIONS

Although the results of the experiments in which adrenal vein blood was collected and assayed on rabbit segments were unequivocal, it seemed desirable to confirm them by other methods. The next experiment (cat 299) is an example of such confirmatory observations made by a method of auto-assay (collection of adrenal blood in a cava pocket for a definite time before and after administration of nicotine, with subsequent release of the blood into the circulation). It was easier to obtain satisfactory curves than with strychnine for reasons already mentioned in the paper on that drug. Some samples of the blood pressure tracings are reproduced in figures 25 to 28. The animal, a male cat weighing 4.18 kgm. (the adrenals weighed 0.761 gram) was anesthetized with urethane. A "long" cava pocket was formed, the abdominal aorta, but not the intestinal arteries, being tied. A blood pressure tracing was taken from a carotid. Numerous pocket experiments were made to determine the output of epinephrin and the effect of nicotine upon it. At 5 (fig. 25) is shown

the effect of the adrenal blood collected for two minutes before nicotine, at 9, the effect of the adrenal blood collected for the same time after intravenous injection of 1 mgm. of nicotine. The nicotine was injected immediately after the pocket was closed off. The amount of epinephrin discharged into the circulation at 9 was obviously much greater than at 5. At 11, a two minute pocket was opened and caused a much smaller effect on the blood pressure than was caused by the opening of a pocket of the same duration before the nicotine injection. At this time,

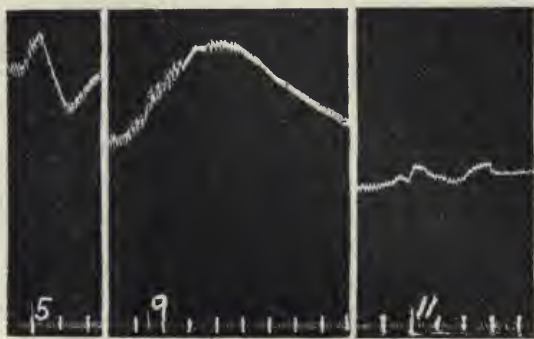


FIG. 25. BLOOD PRESSURE TRACING. CAT 299.

At 5, a two minute pocket (before injecting nicotine) was released; at 9, a two minute pocket, during period of closure of which was injected 1 mgm. nicotine, was released; at 11, a two minute pocket (seven minutes after the nicotine injection) was released. Zero line corresponds with time trace and is moved up 30 mm. and the figure then reduced to two-thirds.

therefore, five to seven minutes after the nicotine injection, the stage of depression was still pronounced.

In figure 26 is shown the effect of releasing a pocket which had been closed for one minute. Immediately after the closure of the pocket, 1 mgm. of nicotine was injected into the jugular vein. This was fourteen minutes after the first injection of nicotine. The rise of pressure, caused by the nicotine, was already over before the pocket was opened, so that the adrenal blood collected in it would contain the increased amount of epinephrin liberated during the transient period of excitation. At 16 the

pocket was opened. The effect on the blood pressure was less than that produced by 0.5 cc. of a 1:33,000 solution of adrenalin injected between 21 and 22, greater than that produced by 0.5 cc. of a 1:66,000 solution of adrenalin injected between 19 and 20, much greater than the effect produced by 0.5 cc. of a 1:130,000 solution of adrenalin injected between 17 and 18. Taking the effect as equivalent to that of 0.5 cc. of a 1:50,000 solution of adrenalin, we get 0.01 mgm. per minute for the cat, or 0.0025 mgm. per kilogram per minute, as the output of epinephrin during

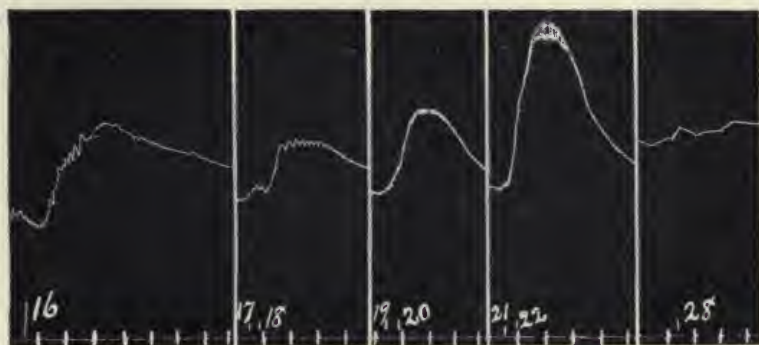


FIG. 26. BLOOD PRESSURE TRACING. CAT 299

At 16, a one minute pocket, during period of closure of which 1 mgm. nicotine was injected, was released; 17 to 18, injected 0.5 cc. of 1:130,000 adrenalin; 19 to 20 injected 0.5 cc. of 1:66,000 adrenalin; 21 to 22, injected 0.5 cc. of 1:33,000 adrenalin; at 23 a one minute pocket was released. Zero line corresponds with time trace and is moved up 22 mm. and the figure then reduced to two-thirds.

the excitation caused by nicotine. This is ten times the normal average in urethanized cats. Probably the increase was still greater in the first half minute after the nicotine injection. It was found, as a matter of fact, that when nicotine was injected intravenously immediately after closing off a pocket, the effect produced on the blood pressure by release of the pocket after one minute, was almost as great as after two minutes, showing that nearly the whole of the epinephrin was liberated in the first minute.

A one-minute pocket, sixteen and one-half minutes after the second nicotine injection (fig. 26, observation 28), caused a very small effect in comparison with the effect at 16, the stage of depression not being entirely recovered from.

A third injection of nicotine was made twenty-three minutes after the second and in the same manner. Although no assay was made of the epinephrin output following this injection the results were qualitatively precisely the same as before, the brief stage of excitation followed by the prolonged depression being equally well marked. The vagi were cut, shortly before the third nicotine injection.

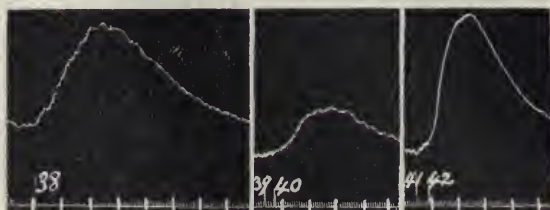


FIG. 27. BLOOD PRESSURE TRACING. CAT 299

At 38 a one minute pocket (during the closure of which 1 mgm. of nicotine was injected) was released; 39 to 40, injected 0.5 cc. of 1: 66,000 adrenalin; 41 to 42 injected 0.5 cc. 1: 33,000 adrenalin. Zero line corresponds with time trace and is moved up 30 mm. and the figure then reduced to two-thirds.

A fourth injection of 1 mgm. of nicotine was made nine minutes after the third, immediately after clipping off a pocket, which was released at 38 (fig. 27) after having been closed for one minute. The rise of pressure produced was much greater than that caused by 0.5 cc. of 1: 66,000 adrenalin (fig. 27, observations 39 and 40) slightly less, as regards the maximum rise, but of greater duration than the rise caused by 0.5 cc. of 1: 33,000 adrenalin. If the epinephrin liberated in one minute under the influence of nicotine be taken as equivalent to 0.5 cc. of a 1: 40,000 solution of adrenalin, we get an output of 0.0125 mgm. per minute for the cat, or 0.003 mgm. per kilogram per minute, i.e., twelve times the average normal output.

Half an hour after the fourth injection of nicotine, when the blood pressure had fallen to about 50 mm. of mercury, and the curve was maintaining a uniform level, favorable for the detection of small epinephrin effects, a fifth injection of 1 mgm. was made immediately after the closure of the cava pocket. After being closed for two minutes the pocket was opened at 63 (fig. 28). The rise of pressure was much less than that produced by the release of a one minute pocket earlier in the experiment. But it has already been pointed out that this proves nothing at all as to a decrease in the amount of epinephrin present unless it be known that the sensitiveness of the test object to

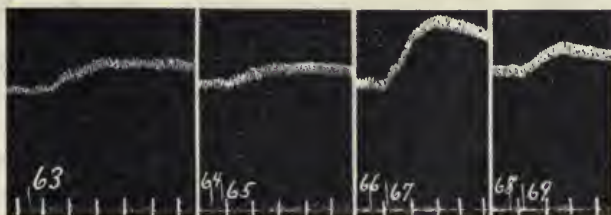


FIG. 28. BLOOD PRESSURE TRACING. CAT 299

At 63 a two minute pocket (during closure of which 1 mgm. of nicotine was injected) was released; 64 to 65, injected 0.5 cc. of 1:66,000 adrenalin; 66 to 67, injected 0.5 cc. of 1:33,000 adrenalin; 68 to 69 injected 0.5 cc. of 1:47,000 adrenalin. Zero line corresponds with time trace. (Reduced to two-thirds).

epinephrin has not decreased. In this case the sensitiveness had decreased decidedly. And the assay showed that a considerable transient augmentation of the epinephrin output had in reality been caused by the nicotine. For the effect was markedly greater than that produced by 0.5 cc. of 1:66,000 adrenalin, and not much different from that caused by 0.5 cc. of 1:47,000 adrenalin (fig. 28), corresponding to an output of 0.01 mgm. for the cat for the two minutes. This is practically the same as for one minute in the previous observations, and if it be remembered that the nicotine excitation effect almost certainly did not endure beyond one minute and probably not so long, it will be seen that even at this stage in the experiment and after so many

doses of nicotine, the transient augmentation of epinephrin output was still little, if at all, inferior to that obtained earlier. The long-lasting stage of depression followed regularly as before. During this stage several injections of strychnine (in all 2.5 mgm.) caused no demonstrable increase in the epinephrin output. Although no great weight can be attached to such a negative result obtained near the close of the experiment, it is altogether in harmony with our conception that strychnine in augmenting the epinephrin output acts upon the central mechanism. When the conductivity of the efferent path is depressed by nicotine, the central stimulation cannot make itself felt.

The results obtained by the above method of auto-assay, entirely corroborated the experiments in which the adrenal blood was assayed on rabbit segments, as to the course, duration and magnitude of the depressant and excitatory actions of nicotine upon the epinephrin output.

EXPERIMENTS ON (DENERVATED) EYE REACTIONS

To obtain corroboration by yet another method, an experiment was made to determine whether any difference existed between the eye reactions, after excision of the superior cervical ganglion, in an otherwise normal cat and in a cat whose epinephrin output had been interfered with by excision of one adrenal and denervation of the other.

Condensed protocol. Cat 300, male, weight 2.7 kgm.

Left superior cervical ganglion excised 44 days previously.

10.00 a.m. Left pupil contracted and nictitating membrane forward.

10.09 a.m. Injected subcutaneously 0.25 mgm. nicotine. The only symptom elicited was slight salivation.

10.48 a.m. Injected subcutaneously 2.5 mgm. nicotine. In 2 to 3 minutes, micturition, defaecation and vomiting occurred; the left pupil became maximally dilated, right dilating moderately.

11.00 a.m. Vomited; the left pupil dilated to maximal, but returned to previous state shortly after the paroxysm of vomiting.

- 11.02 a.m. Cat lying in cage quietly; the left pupil slightly wider than the right.
- 11.25 a.m. The pupils are equal; on exciting the cat, the left becomes wider than right, both dilating somewhat, but equality is soon again established.
- 12.00 m. Pupils equal; excitation causes the same phenomena noted at 11.25.

Condensed protocol. Cat 304, female, weight, 2.15 kgm.

Right adrenal excised; left adrenal denervated; left superior cervical ganglion excised; 46 days before the experiment. Condition excellent.

- 10.00 a.m. Left pupil contracted and nictitating membrane forward.
- 10.08 a.m. Injected subcutaneously 2.5 mgm. nicotine.
- 10.16 a.m. Vomited; during the vomiting paroxysm, both pupils dilated, but the left remained smaller than the right.
- 10.20 a.m. Vomited; pupils as in 10.16 observation.
- 10.25 a.m. Lachrymation, twitching of ears, salivation and erection of hair on tail.
- 11.40 a.m. Injected subcutaneously 2.5 mgm. nicotine.
- 11.42 a.m. Violent vomiting paroxysm; both pupils dilated, the left becoming slightly wider than the right and the left nictitating membrane retracting.
- 11.44 a.m. Left pupil still slightly wider than the right and left nictitating membrane still retracted.
- 11.45 a.m. Vomited; same phenomena as at 11.42.
- 12.30 p.m. Anesthetized with ether; obtained a specimen of jugular blood, then made cava pocket and collected adrenal blood specimens.
- 1.05 p.m. 1st specimen, 1.2 grams in 30 seconds (2.4 grams per minute)
2nd specimen, 6.05 grams in 240 seconds (1.5 grams per minute). 3rd specimen, 3.6 grams in 240 seconds (0.9 gram per minute).

Obtained a specimen of arterial blood.

It was recognized, as already remarked, that such comparisons yield information far less precise and far more difficult of interpretation than the other methods as to the influence of given conditions upon the epinephrin output. Nevertheless, as illustrated in the condensed protocols, a much greater and more durable dilatation of the pupil on the side from which the superior cervical ganglion had been removed, as compared with the normal pupil, was associated with the nicotine action in the nor-

mal cat (cat 300) than in the other (cat 304). Whether this was due to increased epinephrin liberation during the transient stage of excitation, renewed possibly more than once in the non-anesthetized animal as absorption of the poison proceeded, cannot be definitely known, although we have evidence that a maximal dilatation of the pupil of the denervated eye, once established by a large dose of epinephrin, is far more durable than a smaller dilatation, or than a maximal dilatation due to a smaller dose. Naturally under such complicated conditions the depressant action of the drug upon the epinephrin secretion can be studied, if at all, with great difficulty. For the pupil dilatation due to a single dose of adrenalin more than sufficient to produce a maximal effect, does not soon disappear even when all further access of epinephrin is prevented, although by quantitative comparisons under proper conditions it can be shown that the continuous normal discharge of epinephrin after the maximal dilatation has been produced delays measurably the return of the pupil to its original size. Paralysis of the ciliary ganglion, as Langley (5) has pointed out in discussing the experiments of Dale and Laidlaw (1) already referred to, may complicate such observations upon the effect of nicotine on the eye reactions. The difference between the normal cat and the cat whose epinephrin liberation had been interfered with in our observations could not, however, be accounted for in this way, since there is no known reason why the ciliary ganglion should have been affected differently in the two animals.

The epinephrin assay of the adrenal blood specimens collected in cat 304, after the observations on the eye had been completed, showed that both the second and the third specimens were much weaker than 1:6,700,000 adrenalin. The second specimen was weaker than 1:26,500,000, somewhat stronger than 1:40,000,000, about the same as 1:37,000,000, corresponding to an output of 0.00004 mgm. per minute for the cat, or 0.000018 mgm. per kilogram per minute. The third specimen was weaker than 1:20,000,000, much stronger than 1:66,000,000, about the same as 1:26,500,000, corresponding to an output of 0.000034 mgm. per minute for the cat, or 0.000015 mgm. per kilogram per minute, not more than one-sixteenth of the normal average output.

EFFECT OF NICOTINE ON THE EPINEPHRIN STORE

One experiment was made to determine whether nicotine had any detectable influence upon the amount of the epinephrin store of the adrenals. As the superior cervical ganglion had been previously excised on one side, the opportunity was taken to study the eye reactions in this cat also (cat 301) and for this reason the condensed protocol is given.

Condensed protocol. Cat 301; male; weight, 1.65 kgm.

Left adrenal denervated and left superior cervical ganglion excised nineteen days before the experiment.

11.00 a.m. Left pupil contracted and nictitating forward.

11.10 a.m. Injected subcutaneously 2 mgm. nicotine; in about two minutes the cat vomited.

11.18 a.m. Vomited; left pupil only slightly wider than the right.

11.27 a.m. Injected subcutaneously 2 mgm. nicotine.

11.30 a.m. Vomited; left pupil became maximally dilated, right moderately dilated.

11.33 a.m. Left pupil maximal.

11.40 a.m. Right pupil dilated to half the maximal; left pupil maximal; both nictitating membranes are partly forward.

12.10 p.m. Pupils equal (dilated to about one-fourth maximal), nictitating membranes forward.

1.18 p.m. Injected 2 mgm. nicotine.

1.22 p.m. Vomited; left pupil became much wider than right; after vomiting paroxysm the left came down to nearly the width of the right, but remained somewhat wider; the nictitating membrane remained forward. This dose did not cause as great an effect on the pupil as the previous doses.

2.35 p.m. Pupils equal. Injected subcutaneously 2.5 mgm. nicotine.

2.40 p.m. Vomiting paroxysm accompanied by transient dilatation of both pupils, the left becoming slightly wider than the right, but returning to equality very soon.

4.00 p.m. Injected 5 mgm. nicotine; same phenomena as at 1.22; twitching of ears present for the past hour.

4.45 p.m. Killed suddenly and removed adrenals.

Left adrenal weighed 0.15 gm. and contained 0.21 mgm. epinephrin; right adrenal weighed 0.165 grams and contained 0.21 mgm. epinephrin.

No change whatever was demonstrated in the epinephrin store, although the very large amount of 13.5 mgm. of nicotine was injected in 5 doses and the cat was under its influence for five and one-half hours.

SUMMARY

1. The predominant and by far the most durable action of nicotine, whether administered intravenously or hypodermically, upon the epinephrin output is a depressant or paralyzing action. The maximum diminution of the epinephrin output is rather rapidly reached and then there is a more gradual recovery, which when the dose is not too large, proceeds till the original output is approximately attained. At the time of maximum depression no epinephrin at all may be detected in the adrenal vein blood by the test objects chiefly employed (rabbit intestine and uterus segments).

2. The depressant action is preceded by a transient stage of excitation, lasting as a rule in these experiments not longer than from half a minute or less to a minute. In this stage the rate of epinephrin output is markedly increased (from two or three to ten or fifteen times the original output or even more, under our experimental conditions).

3. The latent period of the transient excitation, with intravenous injection of the drug, is very short. In some of the experiments there was evidence that it could not have exceeded a few seconds.

4. The brief stage of excitation passes rather abruptly into the much more durable stage of depression. The maximum increase in the rate of epinephrin output is followed at a relatively short interval by the maximum depression of the rate, after which begins the gradual recovery.

5. The changes in the rate of epinephrin output are roughly parallel to the changes in the blood pressure caused by nicotine, indicating that when the sympathetic ganglion cells on the efferent vasomotor path are being stimulated or depressed, a corresponding stimulation or depression is being exerted on the efferent adrenal secretory path.

6. It may be pointed out that the nicotine effect on the epinephrin output is, speaking generally, the converse of the strychnine effect (see paper 1 of this series). The predominant action of strychnine is a marked and long lasting augmentation of the epinephrin output. There are indications that the strychnine stimulation of the output may be preceded by a brief depression. The nicotine action develops more suddenly than the strychnine action, as might be expected from the fact that the point of attack of nicotine is the efferent path, of strychnine the central mechanism.

7. The transient augmentation of the epinephrin output by nicotine may be associated with an increase in the concentration of epinephrin in the adrenal vein blood much beyond the maximum seen with the slowest blood flows in animals simply anesthetized (with ether, morphine or urethane). The strychnine augmentation of the output has not been observed to be associated with any increase in the normal maximum concentration (something like 1: 500,000 in the serum of adrenal blood, assayed with rabbit segments).

8. Confirmatory evidence of the conclusions deduced from assays of the adrenal blood on rabbit intestine (and uterus) segments has been obtained by a method of auto-assay (collection of adrenal blood for a given time in a cava pocket and study of the blood pressure reactions elicited when the blood is released from the pocket into the circulation), and by other methods.

9. In the one experiment performed the epinephrin store of the adrenals was not found to be altered by nicotine.

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THE ACTION OF DRUGS ON THE OUTPUT OF EPINEPHRIN FROM THE ADRENALS

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INTRODUCTION

The statement occasionally met with in the literature, that strophanthin causes a decided increase in the rate of liberation of epinephrin from the adrenals, has no real experimental basis. Gley (1) mentions strophanthin along with nicotine and anagyrin in speaking of the mode of action of drugs which markedly increase the blood pressure after the bulbospinal centers have been eliminated. But while he cites experiments which he interprets, erroneously we believe (2), as indicating that practically the whole (peripheral) effect of nicotine (and anagyrin) is due to an augmenting influence upon the epinephrin output, he does not state that he made any observations upon strophanthin.

Richards and Wood (3) came to the conclusion that "the intravenous injection of strophanthin is regularly followed by the development in the blood of the capacity to cause decrease of tonus and inhibition of contractions in an isolated strip of intestinal muscle in a manner indistinguishable from that possessed by epinephrine." They obviously consider that their experiments demonstrate a markedly increased rate of epinephrin output under the influence of strophanthin. In reality, however, they show at most that the concentration of epinephrin in blood collected from the inferior vena cava above the level of the

adrenals after strophanthin is greater than before strophanthin. The method used by them (collection of cava blood by a catheter) did not permit the estimation of changes in the rate of the blood flow, and it is clear that if the blood flow was diminished at the time a sample was collected, the concentration must necessarily have been increased, even if no change whatever had occurred in the rate of epinephrin output. It is certain that the blood flow in the cava must often have been diminished at the time the samples after strophanthin injection were collected. Indeed, it is stated that samples were sometimes taken when the animal was dying and in one case a sample was taken after the heart had stopped. Such specimens of blood would necessarily contain epinephrin in a greater concentration than specimens collected with a good flow, without the administration of any drug. No quantitative estimations of the epinephrin output before and after the injection of strophanthin were made, nor was it possible to make them with the method employed. It ought to be explained that these observers were fully aware that the method was defective and only adopted it, so to say, under protest. They point out "that this method is highly faulty in that the blood from the suprarenals is diluted with that from all of the structures whose veins enter the cava below the suprarenal." This is a serious fault, but the really fatal objection is that no provision is made for the measurement of the rate of blood flow. Even with samples collected while the arterial pressure was raised by strophanthin, it is impossible to assume that the blood flow in the upper part of the cava was not reduced. For the vasoconstriction on which the increase of arterial pressure mainly depends may be expected to diminish the rate of the venous flow.

Our experiments were made principally on cats because we have now a large statistical material from which to calculate the normal epinephrin output in this animal, under given experimental conditions, without the administration of any drug except the ordinary anesthetics. It is, therefore, easy to compare in any case an epinephrin output observed after a drug, with the average normal output. Of course comparison of the

output before and after the administration of the drug was made in each experiment and this is the essential thing. But it is often of value to know in addition whether a given result lies within the normal range or clearly surpasses the normal maximum, or falls decidedly below the normal minimum.

Technique. The technique has already been described sufficiently in previous papers. The cats were anesthetized with ether, except in two cases, where a blood pressure assay was to be made, when to ensure a steadier blood pressure curve urethane was employed. In one dog the cerebral peduncles were divided under ether and thereafter no anesthetic was given. But for reasons which will be given more fully in a succeeding paper, although some of them may be indicated in discussing this experiment, we consider that as a rule such mutilations of the central nervous system introduce serious complications in work on the epinephrin output. To practice them for the purpose of avoiding a supposed action of anesthetics upon the output, is, we believe, in general to choose the greater in preference to a lesser, if not an imaginary evil.

As in our previous work, we relied chiefly upon experiments in which blood was collected directly from the adrenals by cannulae in a pocket of the inferior cava. The blood specimens were at once placed on ice, and assayed on rabbit intestine (and uterus) segments. Corroborative evidence was sought by blood pressure auto-assays (collection of adrenal blood in a cava pocket for a given time with estimation of the amount of epinephrin from the blood pressure reaction when the pocket was opened).

The strophanthin (labelled Merck, U. S. P., ix), made up in salt solution to such a concentration that about 0.5 cc. corresponded to the dose to be given was injected into the jugular vein and washed in with 2 cc. of salt solution. It was usually put in more rapidly than in the experiments of Richards and Wood, as our experience with nicotine (2) showed that a transient, though relatively intense stimulation of the epinephrin output by that drug was completely missed when collection of the adrenal blood was delayed beyond half a minute to a minute after the beginning of the injection. We were not generally able to use as large doses as Richards and Wood without killing the animal before satisfactory adrenal blood samples could be procured. This was only partly due to the more rapid injection. For in 5 experiments in which the drug was injected slowly one cat died almost

immediately after the gradual administration of 0.07 mgm. per kilogram during 10 minutes, another after the administration of 0.046 mgm. per kilogram during 10 minutes. The other three cats received respectively 0.05 mgm. per kilogram during $12\frac{1}{2}$ minutes, 0.043 mgm. per kilogram during $12\frac{1}{2}$ minutes, and 0.045 mgm. per kilogram during $12\frac{1}{2}$ minutes. The last cat was under urethane, the others under ether. The blood pressure, which in all cases was somewhat increased during the injection of the strophanthin, remained at a satisfactory level in these three animals while the specimens of adrenal blood were being collected. It was demonstrated by an additional small dose at the end of the experiment that the doses given originally were as great as could possibly be employed. Since in forming the cava pocket in our experiments, the abdominal aorta was always tied near the bifurcation, the nominal dose per kilogram of animal may be somewhat less than the real dose. The point is of no importance in itself and we have entered into these details merely to make it clear that the results which we obtained were not due to the employment of doses below the minimum effective dose. We are certain that the doses were pushed to the utmost limit consistent with the successful carrying out of the observations.

The results of the experiments in which a relatively large dose was injected slowly did not differ noticeably from those of the other experiments, unless, indeed, in being more consistently negative as regards any demonstrable effect of the drug upon the epinephrin output.

EXPERIMENTS WITH DIRECT COLLECTION OF ADRENAL BLOOD AND EPINEPHRIN ASSAY ON RABBIT SEGMENTS

In the first experiment to be quoted, the dose was intermediate between the largest and smallest doses employed in the investigation (0.125 mgm. in a 3.86 kgm. cat). The result was negative. No change in the epinephrin output, beyond the limits of error inherent in the method, could be made out.

Condensed protocol. Cat 293; male; weight, 3.865 kgm.

Anesthetized with ether. Obtained specimen of indifferent blood from jugular. Cut vagi. Made cava pocket. Collected adrenal blood.

- 11.01½ a.m. First specimen, 3.4 grams in 30 seconds (6.8 grams per minute).
- 11.02 a.m. Second specimen, 8.8 grams in 90 seconds (5.87 grams per minute). Blood pressure at end of collection of second specimen was 134 mm. of mercury (fig. 1, observation 4).
- 11.09½ a.m. Injected intravenously 0.125 mgm. strophanthin (fig. 1, observations 5 to 6).
- 11.10½ a.m. Third adrenal specimen, 7.5 grams in 60 seconds (7.5 grams per minute).
- 11.11½ a.m. Fourth adrenal specimen, 8.6 grams in 90 seconds (5.7 grams per minute). Blood pressure at beginning of collection of third specimen was 186 mm. of mercury (fig. 1, observation 7); at beginning of collection of fourth specimen, 182 mm. (observation 8); at end of collection of fourth specimen 144 mm. (observation 9).
- 11.25 a.m. Fifth adrenal specimen, 2.35 grams in 30 seconds (4.7 grams per minute).
- 11.25½ a.m. Sixth adrenal specimen, 7.05 grams in 150 seconds (2.8 grams per minute). Blood pressure at beginning of collection of fifth specimen was 130 mm. of mercury (fig. 1, observation 10); at beginning of collection of sixth specimen 117 mm. (observation 11); at end of collection of sixth specimen 74 mm. (observation 12). Obtained another specimen of venous blood. Combined weight of adrenals 0.377 gram.

Figure 1 indicates the points on the blood pressure curve at which the various adrenal specimens were collected. A few samples of the tracings used for the epinephrin assay are reproduced in figures 2 to 4. The tracings are far too numerous to permit the reproduction of the assay of even one experiment completely. The second specimen, collected before administration of strophanthin, was found to be weaker than 1: 6,660,000 adrenalin (observations not reproduced), weaker than 1: 8,000,000, stronger than 1: 12,500,000 (not reproduced), stronger than 1: 10,000,000 (fig. 2). It was finally taken at 1: 9,000,000, corresponding to an output of epinephrin of 0.00065 mgm. per minute for the cat, or 0.00017 mgm. per kilogram per minute.

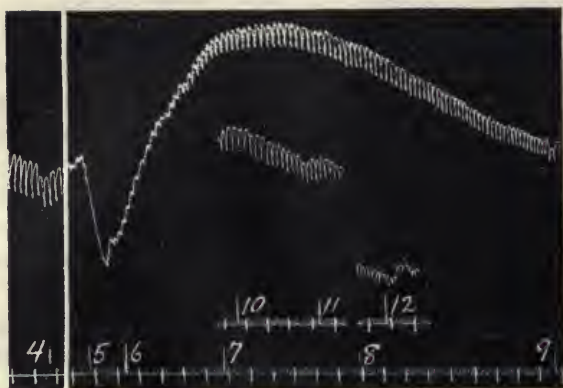


FIG. 1. BLOOD PRESSURE TRACING. CAT 293

4, The end of collection of the second adrenal specimen; 5 to 6, the intravenous injection of strophanthin; 7, the beginning of collection of the third adrenal specimen; 8, beginning of collection of the fourth adrenal specimen; 9, end of collection of the fourth adrenal specimen; 10, beginning of collection of the fifth adrenal specimen; 11, beginning of collection of the 6th adrenal specimen; 12, end of collection of the sixth adrenal specimen. Line of zero pressure corresponds with time trace and is moved up 28 mm. and the figure then reduced to two-thirds. As in all the other blood pressure tracings except when otherwise mentioned, time is marked in seconds and ten seconds.

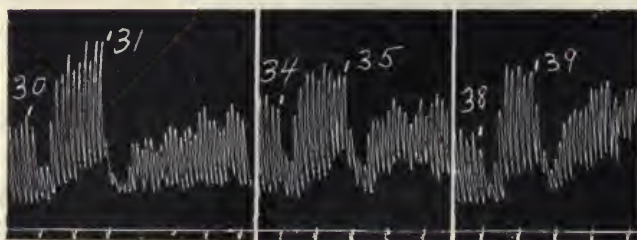


FIG. 2. INTESTINE TRACINGS. BLOODS FROM CAT 293

At 30, 34, and 38, Ringer was replaced by jugular blood and this at 31 by jugular blood to which was added adrenalin to make a concentration of 1:8,000,000; at 35 by the second adrenal specimen (collected before injection of strophanthin); at 39 by jugular blood to which was added adrenalin to make a concentration of 1:10,000,000. All the bloods were diluted with three volumes Ringer (the adrenalin bloods after adding the adrenalin). In all of the intestine and uterus tracings the time is marked in half minutes. Reduced to three-fifths.

The third and fourth specimens were found to be decidedly stronger than 1:10,000,000, but weaker than 1:8,000,000 (fig. 3). The third specimen was found to be much weaker than 1:6,660,000, weaker than 1:8,300,000, and somewhat stronger than 1:9,150,000. The fourth specimen was somewhat stronger than the third (confirmed by other observations), and approxi-

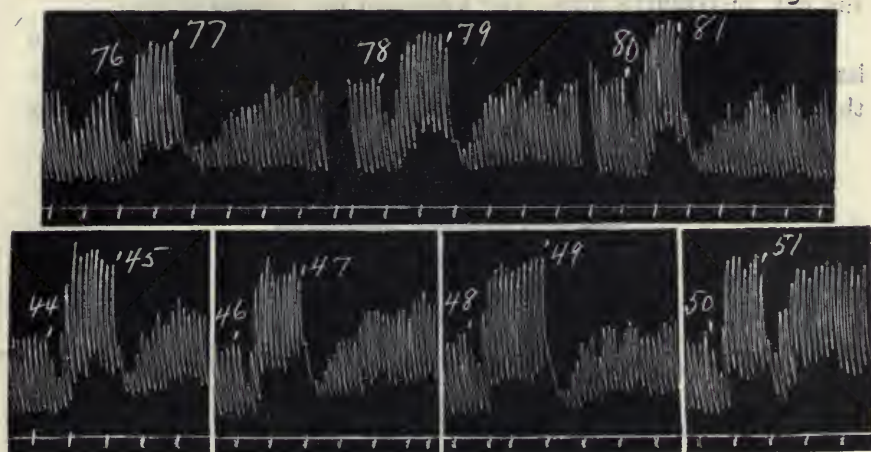


FIG. 3. INTESTINE TRACINGS. BLOODS FROM CAT 293

At 44, 46, 48, 50, 76, 78, and 80, Ringer was replaced by venous blood (collected after the injection of strophanthin); and this at 45 by the third adrenal specimen (collected immediately after the injection of strophanthin); at 47 by the fourth adrenal specimen (collected two minutes after injection of strophanthin); at 49 by venous blood to which was added adrenalin to make a concentration of 1:8,000,000; at 51 by venous blood to which was added adrenalin to make a concentration of 1:10,000,000; at 77 by venous blood to which was added adrenalin to make a concentration of 1:4,100,000; at 79 by venous blood to which was added adrenalin to make a concentration of 1:5,000,000; and at 81 by the sixth adrenal specimen (collected sixteen minutes after injection of strophanthin). All the bloods were diluted with three volumes Ringer (the adrenalin bloods after adding the adrenalin). Reduced to three-fifths.

mately equal to 1:8,300,000 (observations not reproduced). It was confirmed by uterus tracings that the second, third, and fourth specimens were not very different in strength, the fourth being slightly the strongest (fig. 4). Indifferent blood in the same dilution gave only a small increase of tone.

Taking the third specimen (collected one to two minutes after strophanthin injection) at 1:9,000,000, we get for the output of epinephrin 0.0008 mgm. per minute for the cat, or 0.0002 mgm. per kilogram per minute. Taking the fourth specimen (collected two to three and a half minutes after the strophanthin injection) at 1:8,300,000, we get 0.0007 mgm. per minute for the cat, or 0.00018 mgm. per kilogram per minute.

The sixth adrenal specimen, collected sixteen to eighteen and a half minutes after the strophanthin injection, was much stronger than the other specimens, corresponding to the much slower



FIG. 4. UTERUS TRACINGS. BLOODS FROM CAT 293

At 87 Ringer was replaced by the fourth adrenal specimen (collected two minutes after injection of strophanthin); at 88 by the third adrenal specimen (collected immediately after injection of strophanthin); at 89 by the second adrenal specimen (collected before injection of strophanthin). All the bloods were diluted with two volumes Ringer. Reduced to one-half.

blood flow. It was found to be stronger than 1:5,000,000 (fig. 3, observations 79 and 81, confirmed by another pair of observations not reproduced), decidedly weaker than 1:3,300,000 (2 pairs of observations, not reproduced), not very different from 1:4,100,000 (fig. 3, observations 77 and 81). Taking the sixth specimen at 1:4,000,000, we get an epinephrin output of 0.0007 mgm. per minute for the cat, or 0.00017 mgm. per kilogram per minute.

In the next experiment (cat 295) the dose of strophanthin, calculated on the body weight, was two and a half times as

great. The result was the same, no increase in the epinephrin output, beyond the limits of error, was found in an adrenal blood specimen collected two to four minutes after administration of the strophanthin. A more remote specimen could not be obtained as the animal died from the effects of the drug.

Condensed protocol. Cat 295; female; weight, 2.9 kgm.

Anesthetized with ether. Obtained specimen of indifferent blood from jugular. Cut vagi. Made cava pocket. Collected adrenal blood.

- 10.59 a.m. First specimen, 2.2 grams in 30 seconds (4.4 grams per minute).
- 10.59½ a.m. Second specimen, 6.45 grams in 120 seconds (3.2 grams per minute). Blood pressure at end of collection of second specimen was 98 mm. of mercury (fig. 5, observation 4).
- 11.07 a.m. Injected intravenously 0.24 mgm. strophanthin (fig. 5, observations 5 to 6).
- 11.08 a.m. Third adrenal specimen, 5.05 grams in 60 seconds (5.05 grams per minute).
- 11.09 a.m. Fourth adrenal specimen, 5.45 grams in 120 seconds (2.75 grams per minute). Blood pressure at beginning of collection of third specimen was 140 mm. of mercury (fig. 5, observation 7); at beginning of collection of fourth specimen 130 mm. (observation 8); at the end of collection of the fourth specimen 66 mm.

Obtained another specimen of venous blood. Combined weight of adrenals 0.372 gram.

The points on the blood pressure curve at which the adrenal blood samples were procured are given in figure 5. The second specimen, collected before the administration of strophanthin, was shown to be weaker than 1:6,660,000 adrenalin, not far from 1:8,300,000, stronger than 1:10,000,000. It was taken at 1:8,000,000, corresponding to an output of 0.0004 mgm. per minute for the cat, or 0.00014 mgm. per kilogram per minute.

The fourth specimen (beginning two minutes, or allowing for the dead space in cannula and cava, about one and three-quarter



FIG. 5. BLOOD PRESSURE TRACING. CAT 295

4, The end of collection of the second adrenal specimen; 5 to 6, injection of strophanthin; 7, beginning of collection of the third adrenal specimen; 8, beginning of collection of the fourth adrenal specimen. Line of zero pressure corresponds with the time trace and is moved up 20 mm. and the figure then reduced to two-thirds.

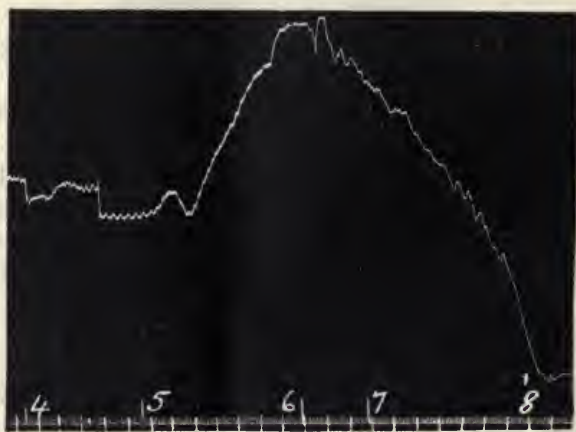


FIG. 6. BLOOD PRESSURE TRACING. CAT 290

4, The end of collection of the second adrenal specimen; 5, injection of strophanthin; 6, beginning of collection of the third specimen; 7, beginning of collection of the fourth specimen; 8, started artificial respiration near the end of collection of fourth specimen. Line of zero pressure corresponds with time trace. Reduced to two-thirds.

minutes after the injection of strophanthin), was shown to be stronger than 1:6,660,000 adrenalin, much weaker than 1:1,660,000, weaker than 1:3,300,000, and not much different from 1:5,000,000. Taking it at 1:5,000,000, we get an output of 0.00055 mgm. per minute for the cat, or 0.00019 mgm. per kilogram per minute.

In the next experiment (cat 290) the largest dose used in the series was given (0.22 mgm. per kilogram). The animal died within three minutes of injection of the strophanthin. The points on the blood pressure curve at which the adrenal blood specimens were procured are indicated in figure 6. The assay revealed no certain increase in epinephrin output in the samples collected after strophanthin, as compared with the original output.

Condensed protocol. Cat 290; female; weight, 2.265 kgm.

Anesthetized with ether. Obtained specimen of indifferent blood from jugular. Cut vagi. Made cava pocket. Collected adrenal blood.

- 11.47½ a.m. First specimen, 0.7 gram in 30 seconds (1.4 grams per minute).
- 11.48 a.m. Second specimen, 3.3 grams in 180 seconds (1.1 grams per minute). Blood pressure at end of collection of second adrenal specimen was 98 mm. of mercury (fig. 6, observation 4).
- 11.56½ a.m. Injected intravenously 0.5 mgm. strophanthin (fig. 6, observation 5).
- 11.57¾ a.m. Third adrenal specimen, 0.65 gram in 30 seconds (1.3 grams per minute).
- 11.58¼ a.m. Fourth adrenal specimen, 0.6 gm. in 90 seconds (0.4 gram per minute). Blood pressure at beginning of collection of third specimen was 156 mm. of mercury (fig. 6, observation 6); at beginning of collection of fourth specimen 135 mm. (observation 7); near the end of collection of fourth specimen, artificial respiration was started (observation 8); blood pressure was 20 mm. The cat was dead at the end of collection of the fourth specimen.

Combined weight of adrenals 0.296 gram.

The second adrenal specimen, taken before strophanthin, was much stronger than 1:4,000,000, stronger than 1:2,750,000 (fig. 7, observations 4, 6 and 8), perhaps somewhat weaker than 1:2,000,000 (fig. 7, observations 8 and 10), but about the same as 1:2,000,000 in another pair of observations (not reproduced). Taking the second specimen at 1:2,000,000, we get 0.00055 mgm. as the output of epinephrin per minute for the cat, or 0.00024 mgm. per kilogram per minute.

The collection of the fourth adrenal specimen was begun one and three-quarter minutes after the injection of strophanthin.

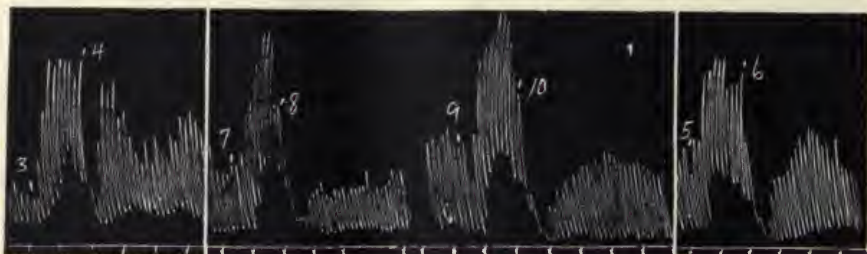


FIG. 7. INTESTINE TRACINGS. BLOODS FROM CAT 290

At 3, 5, 7 and 9, Ringer was replaced by jugular blood and this at 4 by jugular blood to which was added adrenalin to make a concentration of 1:4,000,000; at 6 by jugular blood to which was added adrenalin to make a concentration of 1:2,750,000; at 8 by jugular blood to which was added adrenalin to make a concentration of 1:2,000,000; at 10 by the second adrenal specimen (collected before injection of strophanthin). All the bloods were diluted with three volumes Ringer (the adrenalin bloods after adding the adrenalin). Reduced to one-half.

Allowing for the dead space, this would correspond approximately to the point at which the blood pressure was attaining its maximum. The concentration of epinephrin in the fourth specimen was found to be more than 1:1,300,000, less than 1:700,000, and somewhat less than 1:1,000,000. Taking it at 1:1,100,000, we get 0.00035 mgm. per minute as the epinephrin output for the cat, or 0.00016 mgm. per kilogram per minute. Allowing for the fact that during the last half minute of the nominal period of collection of this specimen the blood pressure was falling most abruptly and that the blood flow had almost

ceased, this is practically the same output as before the drug was administered. The proportion of serum in the blood was 57 per cent as determined by the electrical method, and 56 per cent as determined by the haematocrite (after twenty minutes rotation). The concentration of epinephrin in the serum of the fourth specimen was accordingly about 1:630,000, not much less than the possible normal maximum. Unless strophanthin has the power of causing the epinephrin concentration to rise beyond the possible normal maximum, of which we have seen no clear evidence, the calculated output of epinephrin in this specimen could hardly be expected to show an increase. The experiment is cited mainly to illustrate the point that observations which purport to demonstrate an increased output of epinephrin under the influence of strophanthin merely because after injection of the drug an increased concentration of epinephrin may be found in the blood of the inferior cava, in the absence of information as to changes in the rate of the blood flow, cannot possibly prove what they profess to prove. That epinephrin may be more readily detected in the cava blood at the adrenal level when an animal is dying or even after the heart has stopped than when a good circulation is being maintained is perfectly true, but this has nothing to do with any stimulating action of strophanthin upon the output.

In the next experiment (cat 312) the effect of a much smaller dose (0.0018 mgm. per kilogram) was investigated. This was the smallest dose employed in the series, but it was sufficient to produce a distinct effect upon the blood pressure, a rise succeeded by a progressive fall. The animal was still in good condition when the last adrenal blood sample was procured.

Condensed protocol. Cat 312; female; weight, 2.8 kgm.

Anesthetized with ether. Obtained specimen of indifferent blood from jugular. Cut vagi. Made cava pocket. Collected adrenal blood.

10.22 a.m. First specimen, 1.15 grams in 30 seconds (2.3 grams per minute).

- 10:22½ a.m. Second specimen, 4.3 grams in 180 seconds (1.43 grams per minute). Blood pressure at end of collection of second specimen was 54 mm. of mercury (fig. 8, observation 3).
- 10:29¼ a.m. Injected intravenously 0.05 mgm. strophanthin (fig. 8, observations 4 to 5).
- 10:30 a.m. Third adrenal specimen, 4.15 grams in 60 seconds (4.15 grams per minute).
- 10:31 a.m. Fourth adrenal specimen, 4.95 grams in 120 seconds (2.5 grams per minute).
- 10:33 a.m. Fifth adrenal specimen, 4.1 grams in 180 seconds (1.4 grams per minute). Blood pressure at beginning of collection of third adrenal specimen was 116 mm. of mercury (fig. 8, observation 6); at beginning of collection of fourth specimen 106 mm. (observation 7); at beginning of collection of fifth specimen 74 mm. (observation 8); at end of collection of fifth specimen 52 mm. (observation 9).
- 10:58 a.m. Sixth adrenal specimen, 1.1 grams in 30 seconds (2.2 grams per minute).
- 10:58½ a.m. Seventh adrenal specimen, 5.15 grams in 240 seconds (1.3 grams per minute). Blood pressure at beginning of collection of sixth adrenal specimen was 76 mm. of mercury (fig. 8, observation 10); at beginning of collection of seventh specimen 70 mm. (observation 11); at end of collection of seventh specimen 48 mm. (observation 12).

Obtained another specimen of venous blood. Combined weight of adrenals 0.34 gram.

Figure 8 shows the points on the blood pressure curve at which the adrenal bloods were collected. No evidence was obtained of any increase in the rate of output, with the exception of a possible small increase in the last specimen, collected half an hour after injection of the strophanthin. The initial output, however, before strophanthin was below the average normal output in cats anesthetized by ether, and the output calculated for the last specimen did not at all exceed the normal average. On the other hand, there was a distinct diminution in the output for

the specimen (fourth), collected about two minutes after the administration of the drug. The assay was such that there could be no doubt that at this time the output was depressed for a brief period. This is about the only instance in which such a depression has been noted in this investigation, and we do not know whether it should be attributed to the strophanthin or not. Where an abrupt change in the rate of blood flow is coincident with the collection of a sample, it is obvious that the blood already in the adrenals with the lower concentration of epinephrin corresponding to a greater blood flow will pass into a

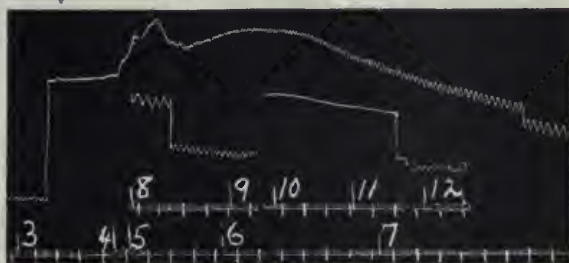


FIG. 8. BLOOD PRESSURE TRACING. CAT 312

3, The end of collection of the second adrenal specimen; 4 to 5, intravenous injection of strophanthin; 6, beginning of collection of the third adrenal specimen; 7, beginning of collection of fourth adrenal specimen; 8, beginning of collection of fifth adrenal specimen; 9, end of collection of fifth adrenal specimen; 10, beginning of collection of sixth adrenal specimen; 11, beginning of collection of seventh adrenal specimen; 12, end of collection of seventh adrenal specimen. Line of zero pressure corresponds with time trace and is moved up 15 mm.

sample collected with a lower average blood flow and will, to some extent, diminish its concentration. Considering, however, the maximum possible amount of blood in the adrenal medulla and the blood flow when the third and fourth specimens were obtained in this experiment, this factor could only play an insignificant part.

A few samples of the tracings used in the epinephrin assay are reproduced in figures 9 to 11. The second specimen, collected before injection of strophanthin, was weaker than 1:3,000,000 adrenalin, stronger than 1:6,000,000 (fig. 9). In other obser-

vations (not reproduced) it was shown that the second specimen was nearer 1:3,000,000 than 1:6,000,000, and not far from 1:4,000,000, corresponding to an output of epinephrin of 0.00035 mgm. per minute for the cat, or about 0.00013 mgm. per kilogram per minute.

The third specimen, collected forty-five seconds, or allowing for the dead space, not more than thirty-five seconds after the injection of strophanthin, was much weaker than 1:3,000,000,

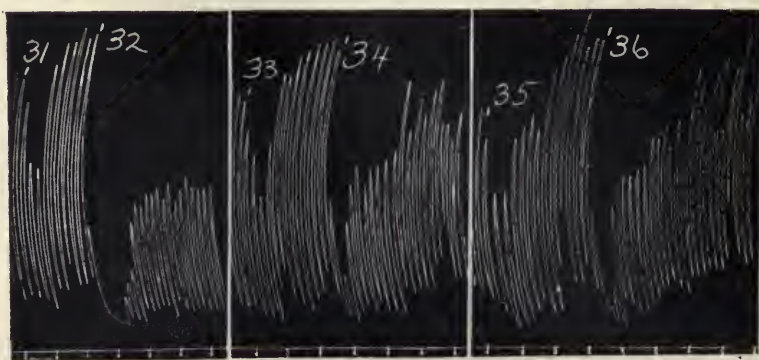


FIG. 9. INTESTINE TRACINGS. BLOODS FROM CAT 312

At 31, 33 and 35 Tyrode's solution was replaced by jugular blood and this at 32 by jugular blood to which was added adrenalin to make a concentration of 1:3,000,000; at 34 by jugular blood to which was added adrenalin to make a concentration of 1:6,000,000; at 36 by the second adrenal specimen (collected before injection of strophanthin). All the bloods were diluted with three volumes Tyrode's solution (the adrenalin bloods after adding the adrenalin). Reduced to one-half.

weaker than 1:6,000,000, somewhat weaker than 1:9,000,000, not far from 1:12,000,000 (fig. 10, observations 62 and 64). Taking it at 1:12,000,000, we get an output of 0.00035 mgm. per minute for the cat, the same as for the second specimen.

The fourth specimen, obtained one and three-quarter minutes, or allowing for the dead space, one and a half minutes after the administration of the drug, was found to be much weaker than the second, and also decidedly weaker than the third specimen (fig. 10, observations 64 and 70). It was much weaker than

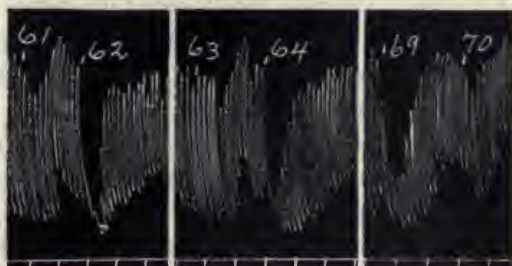


FIG. 10. INTESTINE TRACINGS. BLOODS FROM CAT 312

At 61, 63, and 69, Tyrode's solution was replaced by venous blood and this at 62 by venous blood to which was added adrenalin to make a concentration of 1:12,000,000; at 64 by the third adrenal specimen (collected immediately after injection of strophanthin); at 70 by the fourth adrenal specimen (collected two minutes after injection of strophanthin). All the bloods were diluted with three volumes Tyrode's solution (the adrenalin bloods after adding the adrenalin). Reduced to one-half.

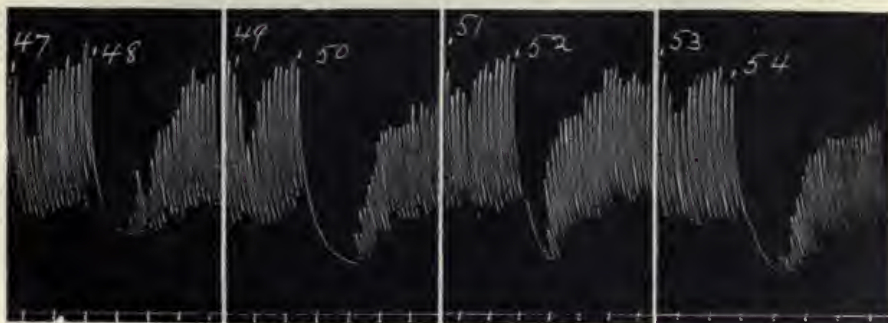


FIG. 11. INTESTINE TRACINGS. BLOODS FROM CAT 312

At 47, 49, 51, and 53 Tyrode's solution was replaced by venous blood and this at 48 by the fifth adrenal specimen (collected four minutes after injection of strophanthin); at 50 by venous blood to which was added adrenalin to make a concentration of 1:2,250,000; at 52 by venous blood to which was added adrenalin to make a concentration of 1:3,750,000, at 54 by the seventh adrenal specimen (collected thirty minutes after injection of strophanthin). All the bloods were diluted with three volumes Tyrode's solution (the adrenalin bloods after adding the adrenalin). Reduced to one-half.

1:9,000,000 adrenalin (shown by 2 separate pairs of observations), and probably weaker than 1:18,000,000, corresponding to an output of not more than 0.00014 mgm. per minute for the cat, or 0.00005 mgm. per kilogram per minute.

The fifth adrenal specimen, collected from the fourth to the seventh minute after strophanthin, was shown to be much stronger than the third specimen, weaker than 1:2,250,000 adrenalin (fig. 11, observations 48 and 50), somewhat stronger than 1:3,750,000 (fig. 11, observations 48 and 52). It was taken at 1:3,500,000, corresponding to an output of 0.0004 mgm. per minute for the cat, or 0.00014 mgm. per kilogram per minute. Up to this point then no increase had occurred in the epinephrin output.

The seventh specimen, procured half an hour after injection of strophanthin, was decidedly stronger than 1:3,750,000 adrenalin (fig. 11, observations 52 and 54) and than the fifth specimen, not far from 1:2,250,000 (fig. 11, observations 50 and 54), corresponding to an output of 0.00055 mgm. per minute for the cat, or 0.0002 mgm. per kilogram per minute.

The next experiment (cat 294) is cited because here too there appeared to be a somewhat increased output, although not beyond the normal range, in a remote adrenal blood specimen (collected twenty minutes after strophanthin injection), whereas the specimens collected within a few minutes of the administration of the drug showed no increase.

Condensed protocol. Cat 294; male; weight, 2.73 kgm.

Anesthetized with ether. Obtained specimen of indifferent blood from jugular. Cut vagi. Made cava pocket. Collected adrenal blood.

11.42½ a.m. First specimen, 2.65 grams in 30 seconds (5.3 grams per minute).

11.43 a.m. Second specimen, 6.85 grams in 90 seconds (4.8 grams per minute). Blood pressure at end of collection of second adrenal specimen was 109 mm. of mercury (fig. 12, observation 4).

11.50 a.m. Injected intravenously 0.15 mgm. strophanthin (fig. 12, observations 5 to 6).

- 11.51 $\frac{3}{4}$ a.m. Third adrenal specimen, 5.3 grams in 60 seconds (5.3 grams per minute).
- 11.52 $\frac{3}{4}$ a.m. Fourth adrenal specimen, 6.2 grams in 120 seconds (3.1 grams per minute). Blood pressure at beginning of collection of third specimen was 142 mm. of mercury (fig. 12, observation 7); at beginning of collection of 4th specimen 124 mm. (observation 8); at end of collection of fourth specimen 80 mm. (observation 9).
- 12.09 p.m. Fifth adrenal specimen, 1.3 grams in 30 seconds (2.6 grams per minute).



FIG. 12. BLOOD PRESSURE TRACING. CAT 294

4, The end of collection of the second adrenal specimen; 5 to 6, intravenous injection of strophanthin; 7, beginning of collection of third adrenal specimen; 8, beginning of collection of fourth adrenal specimen; 9, end of collection of fourth adrenal specimen; 10, beginning of collection of fifth adrenal specimen; 11, beginning of collection of sixth adrenal specimen; 12, end of collection of sixth adrenal specimen. Line of zero pressure corresponds with time trace and is moved up 18 mm. and the figure then reduced to two-thirds.

- 12.09 $\frac{1}{2}$ p.m. Sixth adrenal specimen, 4.5 grams in 180 seconds (1.5 grams per minute). Blood pressure at beginning of collection of fifth adrenal specimen was 98 mm. of mercury (fig. 12, observation 10); at beginning of collection of sixth specimen 78 mm. (observation 11); at end of collection of sixth specimen 60 mm. (observation 12).

Obtained another specimen of venous blood. Combined weight of adrenals 0.316 gram.

The points on the blood pressure curve at which the adrenal specimens were procured are indicated in figure 12.

The assay showed that the second specimen, collected before strophanthin, was stronger than 1:8,300,000 adrenalin, weaker than 1:5,000,000, weaker than 1:6,000,000. It was taken at 1:7,500,000, equivalent to an epinephrin output of 0.00065 mgm. per minute for the cat, or 0.00024 mgm. per kilogram per minute.

The third specimen, obtained about a minute after the end of the strophanthin injection, was weaker than 1:6,660,000, somewhat weaker than 1:8,300,000, somewhat stronger than 1:10,000,000. It was taken at 1:9,000,000, corresponding to an output of 0.006 mgm. per minute for the cat, or 0.00022 mgm. per kilogram per minute.

The fourth specimen, collected from two to four minutes after the end of the strophanthin injection, was found to be weaker than 1:5,000,000 adrenalin, probably slightly weaker than 1:6,000,000. It was taken at 1:6,200,000, equivalent to an output of 0.0005 mgm. per minute for the cat, or 0.0018 mgm. per kilogram per minute.

The sixth specimen, collected nineteen to twenty-two minutes after the administration of the drug, when the blood flow was much less than in the case of the other specimens, although still fair, was found to be stronger than 1:1,660,000 adrenalin, slightly stronger than 1:1,250,000, weaker than 1:830,000. It was confirmed by uterus tracings that the sixth specimen was much stronger than the third and fourth. Taking its concentration at 1:1,200,000, we get an output of 0.00125 mgm. per minute for the cat, or 0.00045 mgm. per kilogram per minute, nearly double the output at the time of collection of the second specimen, although not beyond the maximum output observed in anesthetized cats. We believe it would be unwarrantable to conclude that this small and remote effect was occasioned by a direct excitation of the secretion by strophanthin, seeing that in the earlier specimens at the time the drug was causing a decided vasoconstrictor stimulation, there was no increase whatever. It seems likely that a small and inconstant increase in the rate of liberation appearing a long time after administration of a drug is due to some general toxic action rather than to stimulation of

a secretory mechanism notable for the promptitude with which it responds to appropriate excitation.

This suggestion is supported by the fact that whenever an apparent small increase has been seen in the output in adrenal blood specimens collected soon after the injection of strophanthin, the dose has been such as to kill the animal very quickly thereafter. For example, in cat 296, although the dose (0.05 mgm. per kilogram) was slightly smaller than in cat 290, the animal died very soon after the end of collection of the fourth specimen, four to five minutes after the administration of the drug. A moderate increase in the output was found, both in the third and fourth specimens, although it was still well within the normal range. The assay was such that an increase of less than 50 per cent could be certainly demonstrated and the calculated increase was somewhat more than this. There is no doubt then that there was a small increase in the output in this experiment, beginning half a minute to a minute after the injection of the drug and still present two to four minutes after the injection. But compared with the augmentation caused by strychnine or the transient augmentation caused by nicotine, the effect, even if in this experiment it was directly due to the strophanthin, is insignificant.

Condensed protocol. Cat 296; female; weight, 2.0 kgm.

Anesthetized with ether. Obtained specimen of indifferent blood from jugular. Cut vagi. Made cava pocket. Collected adrenal blood.

10.40 a.m. First specimen, small—not weighed.

10.40½ a.m. Second specimen, 3.7 grams in 240 seconds (0.92 gram per minute). Blood pressure at beginning of collection of second adrenal specimen was 74 mm. of mercury (fig. 13, observation 3); at the end of collection of second specimen 64 mm. (observation 4).

10.51 a.m. Injected intravenously 0.1 mgm. strophanthin (fig. 13, observations 5 to 6).

10.51½ a.m. Third adrenal specimen, 2.6 grams in 90 seconds (1.7 grams per minute).

10.53 a.m. Fourth adrenal specimen, 2.85 grams in 150 seconds (1.14 grams per minute). Blood pressure at beginning of collection of third specimen was 100 mm. of mercury (fig. 13, observation 7); at beginning of collection of fourth specimen 82 mm. (observation 8); at end of collection of fourth specimen 56 mm. (observation 9). Shortly after the collection of fourth adrenal specimen the heart became irregular and soon stopped.

Combined weight of adrenals 0.215 gram.

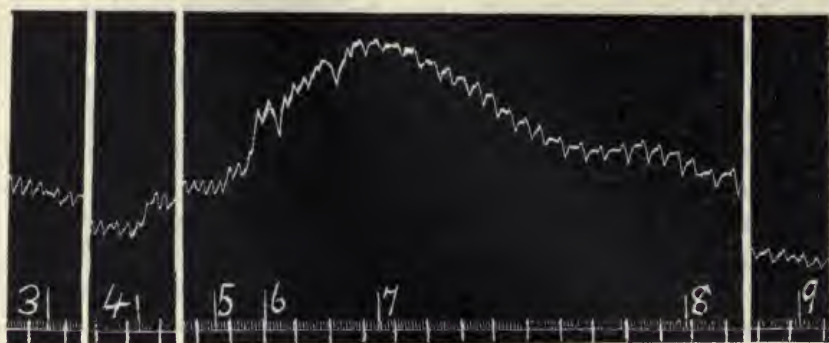


FIG. 13. BLOOD PRESSURE TRACING. CAT 296

3 and 4 The beginning and end of collection of second adrenal specimen; 5 to 6, intravenous injection of strophanthin; 7, beginning of collection of third adrenal specimen; 8, beginning of collection of fourth adrenal specimen; 9, end of collection of fourth adrenal specimen. Line of zero pressure corresponds with time trace and is moved up 18 mm.

Figure 13 shows the blood pressure at the time the adrenal blood specimens were procured. In figures 14 to 16 are reproduced some of the tracings used in the assay. It was shown that the second specimen, collected before injection of strophanthin, was weaker than 1:1,660,000 adrenalin, weaker than 1:2,100,000, stronger than 1:2,500,000 (fig. 14, confirmed by other observations not reproduced). It was taken at 1:2,300,000, corresponding to an output of 0.0004 mgm. per minute for the cat, or 0.0002 mgm. per kilogram per minute.

The third specimen was much weaker than 1:1,660,000, much weaker than the fourth specimen (fig. 15), not very different in

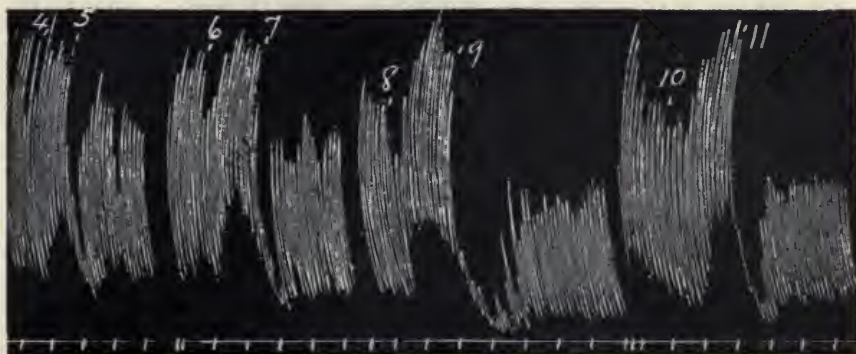


FIG. 14. INTESTINE TRACINGS. BLOODS FROM CAT 296

At 4, 6, 8 and 10, Ringer was replaced by jugular blood; and this at 5 by jugular blood to which was added adrenalin to make a concentration of 1:2,500,000; at 7 by the second adrenal specimen (collected before injection of strophanthin); at 9 by jugular blood to which was added adrenalin to make a concentration of 1:1,660,000; at 11 by jugular blood to which was added adrenalin to make a concentration of 1:2,100,000. All the bloods were diluted with three volumes Ringer (the adrenalin bloods after adding the adrenalin). Reduced to one-half.

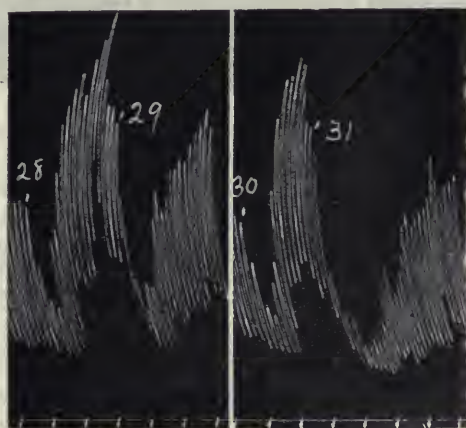


FIG. 15. INTESTINE TRACINGS. BLOODS FROM CAT 296

At 28 and 30 Ringer was replaced by jugular blood to which was added strophanthin (0.001 mgm. to each 1.25 cc. of blood); and this at 29 by the third adrenal specimen (collected immediately after injection of strophanthin); at 31 by the fourth adrenal specimen (collected two minutes after injection of strophanthin). All the bloods were diluted with three volumes Ringer. Reduced to one-half.

concentration from the second specimen, but slightly weaker. It was taken at 1:2,500,000, equivalent to an output of 0.0007 mgm. per minute for the cat, or 0.00035 mgm. per kilogram per minute. The fourth specimen was much stronger than 1:2,500,000, much stronger than the second specimen (fig. 16, observations 19 and 21), little different from 1:1,660,000 (fig. 16, observations 19 and 25, confirmed by other observations not reproduced), but probably slightly stronger. Taking it at 1:1,600,000, we get 0.0007

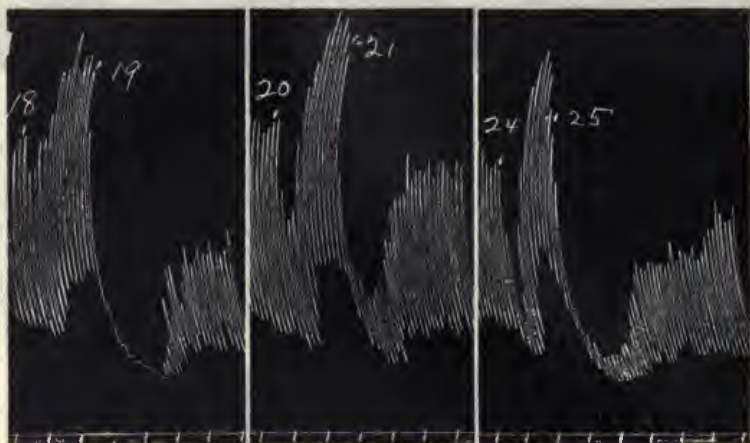


FIG. 16. INTESTINE TRACINGS. BLOODS FROM CAT 296

At 18, 20 and 24 Ringer was replaced by jugular blood and this at 19 by the fourth adrenal specimen (collected two minutes after injection of strophanthin); at 21 by the second adrenal specimen (collected before injection of strophanthin); at 25 by jugular blood to which was added adrenalin to make a concentration of 1:1,660,000. All the bloods were diluted with three volumes Ringer (the adrenalin bloods after adding the adrenalin). Reduced to one-half.

mgm. per minute for the cat, or 0.00035 mgm. per kilogram per minute, the same output as for the third specimen.

The two following protocols (cats 317 and 319) illustrate the observations in which the strophanthin was injected slowly.

Condensed protocol. Cat 317; female; weight, 2.0 kgms.

Anesthetized with ether. Obtained indifferent blood from jugular. Cut vagi. Made cava pocket. Collected adrenal blood.

- 10.10 a.m. First specimen, 2.3 grams in 30 seconds (4.6 grams per minute).
- 10.10½ a.m. Second specimen, 7.65 grams in 2 minutes (3.82 grams per minute). Blood pressure at beginning of collection of second specimen 130 mm. of mercury, at end of collection 110 mm.
- 10.23 to 10.35½ a.m. Injected intravenously 0.1 mgm. strophanthin.
- 10.36½ a.m. Third adrenal specimen, 2.15 grams in 30 seconds (3.05 grams per minute).
- 10.37 a.m. Fourth adrenal specimen, 6.1 grams in 2 minutes (3.05 grams per minute). Blood pressure at beginning of collection of third specimen 136 mm. of mercury; at beginning of collection of fourth specimen 130 mm.; at end of fourth specimen 100 mm.
- 10.53 a.m. Fifth adrenal specimen, 1 gram in 30 seconds (2 grams per minute).
- 10.53½ a.m. Sixth adrenal specimen, 4.1 grams in 3 minutes (1.4 grams per minute). Blood pressure at beginning and end of collection of fifth specimen 76 mm. of mercury; at end of sixth specimen 48 mm.

Weight of adrenals 0.29 gram.

The second specimen, collected before administration of the drug, was found to have a smaller concentration of epinephrin than the fourth, collected at the end of the strophanthin injection, and the fourth, a smaller concentration than the sixth, procured about twenty minutes after the injection. The detailed assay showed that the second specimen was much weaker than 1:2,750,000 adrenalin, decidedly weaker than 1:7,000,000, distinctly stronger than 1:10,000,000 (confirmed by several sets of observations). It was taken at 1:9,000,000, corresponding to an output of 0.00042 mgm. per minute for the cat, or 0.00021 mgm. per kilogram per minute.

The fourth specimen was stronger than 1:7,000,000, weaker than 1:5,500,000, approximately the same as 1:6,000,000, equivalent to an output of 0.0005 mgm. per minute for the cat, or 0.00025 mgm. per kilogram per minute. The sixth specimen was much stronger than 1:5,500,000, much weaker than 1:1,750,000, somewhat weaker than 1:2,750,000. It was taken at 1:3,000,000

corresponding to an output of 0.00047 mgm. per minute for the cat, or 0.00023 mgm. per kilogram per minute. In this animal accordingly no change in the epinephrin output could be demonstrated after strophanthin.

Condensed protocol: Cat 319; male; weight, 2.3 kgms.

Anesthetized with ether. Obtained indifferent blood from jugular. Cut vagi. Made cava pocket. Collected adrenal blood.

10.16½ a.m. First specimen, 1.05 grams in 30 seconds (2.1 grams per minute).

10.17 a.m. Second specimen, 5.1 grams in 180 seconds (1.7 grams per minute). Blood pressure at end of collection of second specimen was 76 mm. of mercury, at beginning 90 mm.

10.27½ a.m. Beginning of injection of 0.1 mgm. strophanthin.

10.40 a.m. End of injection of strophanthin. Blood pressure at end of injection of strophanthin was 112 mm. of mercury.

10.41 a.m. Third adrenal specimen, 1 gram in 30 seconds (2 grams per minute).

10.41½ a.m. Fourth adrenal specimen, 5.45 grams in 240 seconds (1.36 grams per minute). Blood pressure at beginning of collection of third specimen was 94 mm. of mercury; at beginning of collection of fourth specimen 89 mm.; at end of collection of fourth specimen 67 mm.

11.04½ a.m. Fifth adrenal specimen, 30 seconds collection—not weighed.

11.05 a.m. Sixth adrenal specimen, 2.55 grams in 270 seconds (0.57 gram per minute). Blood pressure at beginning of collection of sixth specimen was 59 mm. of mercury; at end of collection 47 mm.

Obtained another specimen of venous blood. Combined weight of adrenals 0.301 gram.

The second specimen, collected before administration of strophanthin, was shown to have a concentration of epinephrin much less than 1:3,750,000 and somewhat less than 1:5,000,000. It was taken at 1:5,750,000, corresponding to an output of

0.0003 mgm. per minute for the cat, or 0.00013 mgm. per kilogram per minute.

The fourth specimen, procured immediately after the end of the strophanthin injection was found to be much weaker than 1:2,500,000, and somewhat weaker than 1:3,750,000 (confirmed by several pairs of observations). It was finally taken at 1:4,000,000, corresponding to an output of 0.00034 mgm. per minute for the cat, or 0.00014 mgm. per kilogram per minute.

The sixth specimen, collected about half an hour after the end of the strophanthin injection was decidedly weaker than 1:1,250,000, and approximately the same as 1:1,875,000 adrenalin (confirmed by several sets of observations). Taking it at 1:1,875,000, we get 0.0003 mgm. per minute as the epinephrin output for the cat, or 0.00013 mgm. per kilogram per minute. Therefore, no change whatever could be demonstrated in this animal after administration of the drug.

The last experiment with rabbit segment assays which will be referred to, was made on a dog (297) in which the brain stem was divided at the cerebral peduncles. This was done because in the experiments of Richards and Wood the usual procedure was to begin with a brain mutilation which rendered the subsequent administration of an anesthetic unnecessary. As already mentioned, however, our experience is that such interference with the central nervous system may introduce such complications that it should be avoided as a routine measure in experiments on the epinephrin output. The experiment to be described is a good illustration of this.

Condensed protocol. Dog 297; female; weight, 5.09 kgm.

Anesthetized with ether. Through a trephine opening cut across the cerebral peduncles and discontinued anesthetic; respirations were good, but for uniformity of the blood pressure curve started artificial respiration. Obtained specimen of indifferent blood from jugular. Made cava pocket. Collected adrenal blood.

10.39 a.m. First specimen, 2.9 grams in 30 seconds (5.8 grams per minute).

- 10.39½ a.m. Second specimen, 8.55 grams in 120 seconds (4.3 grams per minute). Blood pressure at beginning of collection of second adrenal specimen was 55 mm. of mercury.
- 10.47 a.m. to 10.47¾ a.m. Injected intravenously 0.4 mgm. strophanthin in doses of 0.25 mgm. and 0.15 mgm.
- 10.49 a.m. Third adrenal specimen, 4.25 grams in 60 seconds (4.25 grams per minute).
- 10.50 a.m. Fourth adrenal specimen, 6.55 grams in 120 seconds (3.28 grams per minute). Blood pressure at beginning of collection of third adrenal specimen was 57 mm. of mercury; at beginning of collection of fourth specimen 54 mm.; at end of collection of fourth specimen 45 mm.
- 11.02 a.m. Fifth adrenal specimen, 0.85 gram in 60 seconds (0.85 gram per minute).
- 11.03 a.m. Sixth adrenal specimen, 2.3 grams in 240 seconds (0.6 gram per minute). Blood pressure at beginning of collection of fifth adrenal specimen was 34 mm. of mercury; at beginning of collection of sixth specimen 28 mm.

Obtained another specimen of venous blood. Combined weight of adrenals 0.883 gram.

Some of the tracings used in the epinephrin assay are reproduced in figures 17 to 20. The second adrenal blood specimen, collected before the administration of strophanthin, the blood pressure being 55 mm. of mercury, was found to be much stronger than 1:5,000,000 (observations not reproduced), or than 1:2,500,000 (fig. 17, observations 7 and 9), stronger than 1:1,250,000 (fig. 17, observations 9 and 13), and very much the same as 1:830,000 (fig. 17, observations 9 and 11). These results were corroborated by other intestine tracings (not reproduced). It was confirmed by uterus tracings (fig. 18) that the second adrenal specimen was stronger than 1:1,250,000. The indifferent (jugular) blood in the same dilution gave only an insignificant effect on the uterus segment. Taking the second specimen at 1:830,000, we get an epinephrin output of 0.0052 mgm. per minute for the dog, or 0.001 mgm. per kilogram per

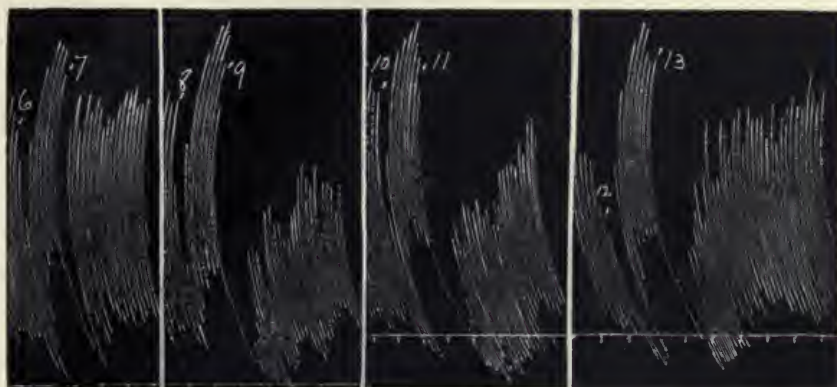


FIG. 17. INTESTINE TRACINGS. BLOODS FROM DOG 297

At 6, 8, 10, and 12 Ringer was replaced by jugular blood and this at 7 by jugular blood to which was added adrenalin to make a concentration of 1:2,500,000; at 9 by the second adrenal specimen (collected before injection of strophanthin); at 11 by jugular blood to which was added adrenalin to make a concentration of 1:830,000; at 13 by jugular blood to which was added adrenalin to make a concentration of 1:1,250,000. All the bloods were diluted with three volumes Ringer (the adrenalin bloods after adding the adrenalin). Reduced to two-fifths.

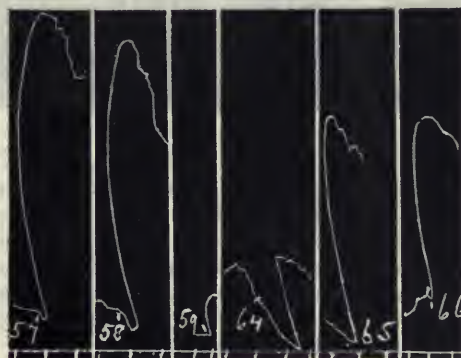


FIG. 18. UTERUS TRACINGS. BLOODS FROM DOG 297

At 57 Ringer was replaced by the second adrenal specimen (collected before injection of strophanthin); at 58 by jugular blood to which was added adrenalin to make a concentration of 1:1,250,000; at 59 and 64 by jugular blood; at 65 by the second adrenal specimen; at 66 by the third adrenal specimen (collected immediately after injection of strophanthin). All the bloods were diluted with ten volumes Ringer (the adrenalin blood after adding the adrenalin). Observations 64, 65 and 66 were taken with a smaller magnification than the other observations. Reduced to one-half.

minute. This is more than four times the average in dogs under ordinary anesthesia (4) and much beyond the maximum range, suggesting that the brain operation either had removed an inhibitory influence on the secretion, exercised by some portion of the brain above the lesion, or that the irritative effects of the lesion had increased the output. We have seen indications in our work on strychnine (5) that this drug may, under certain

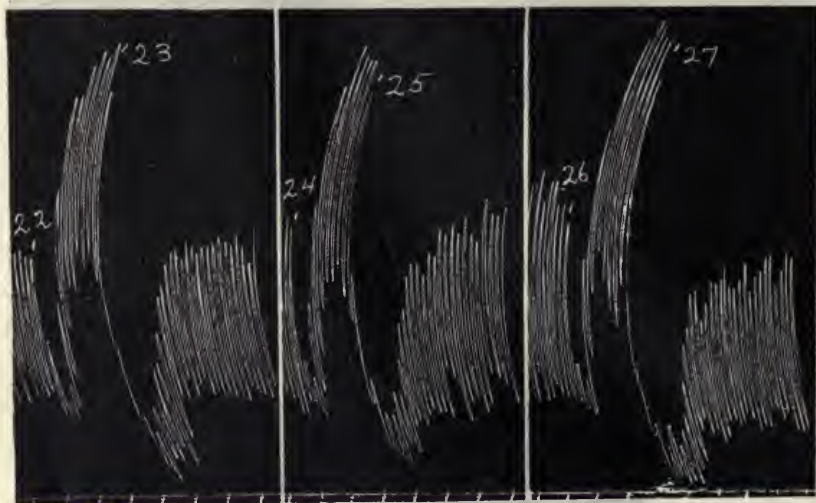


FIG. 19. INTESTINE TRACINGS. BLOODS FROM DOG 297

At 22, 24 and 26 Ringer was replaced by venous blood (collected after injection of strophanthin); and this at 23 by the third adrenal specimen (collected immediately after injection of strophanthin); at 25 by the venous blood to which was added adrenalin to make a concentration of 1: 830,000; at 27 by the venous blood to which was added adrenalin to make a concentration of 1: 660,000. All the bloods were diluted with three volumes Ringer (the adrenalin bloods after adding the adrenalin). Reduced to one-half.

conditions, cause a transient diminution of the epinephrin discharge preceding the marked and prolonged augmentation which is its predominant action, and it is possible that this effect may be due to a brief excitation by strychnine of an inhibitory mechanism situated at a higher level than the center whose stimulation causes the increased liberation. Elliott (6) has

shown that such brain mutilations are associated with a decided diminution in the epinephrin store of the adrenals, and although, as we have several times pointed out, a diminution in the epinephrin store is not of itself evidence that the epinephrin discharge has been augmented, such a diminution in the store may very well, in certain cases, be associated with an increased

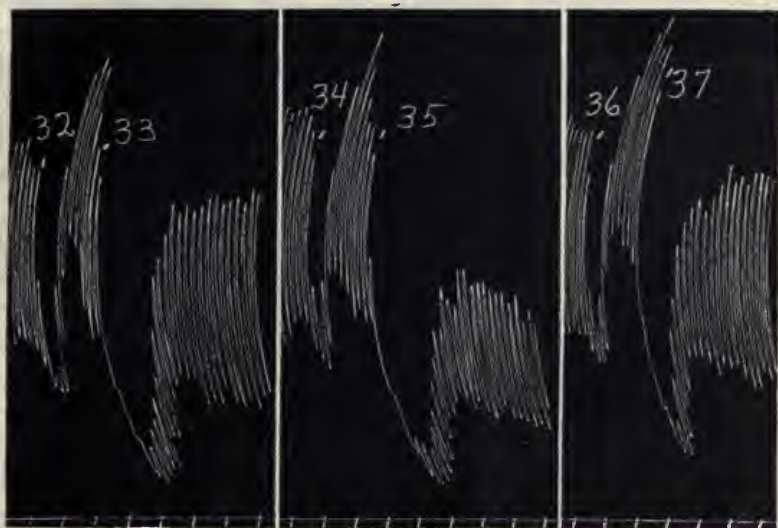


FIG. 20. INTESTINE TRACINGS. BLOODS FROM DOG 297

At 32, 34, and 36 Ringer was replaced by venous blood (collected after injection of strophanthin); and this at 33 by the fourth adrenal specimen (collected two minutes after injection of strophanthin); at 35 by the venous blood to which was added adrenalin to make a concentration of 1: 660,000; at 37 by the venous blood to which was added adrenalin to make a concentration of 1: 830,000. All the bloods were diluted with three volumes Ringer (the adrenalin bloods after adding the adrenalin). Reduced to one-half.

output, due either to removal of an inhibitory influence, or to the production of a continuous stimulation.

The third adrenal specimen, the collection of which was begun a little more than a minute after completion of the strophanthin injection, had practically the same concentration of epinephrin as the second. On the intestine segment it was shown to be much stronger than 1: 1,250,000, stronger than 1: 1,000,000

(confirmed by several observations), probably slightly stronger than 1:830,000 (fig. 19, observations 23 and 25), but decidedly weaker than 1:670,000 (fig. 19, observations 23 and 27). Taking the third specimen at 1:800,000, we get 0.0053 mgm. per minute for the dog, or 0.001 mgm. per kilogram per minute, the same as for the specimen before injection of strophanthin.

The fourth adrenal specimen, beginning about two minutes after the strophanthin injection, had very much the same concentration as the third and the second. It was probably slightly stronger than 1:830,000, but markedly weaker than 1:670,000 (fig. 20). Taking it at 1:800,000, we get an output of 0.00041 mgm. per minute for the dog, or 0.0008 mgm. per kilogram per minute.

The sixth adrenal specimen, obtained fifteen minutes after injection of strophanthin, was found to be decidedly stronger than 1:830,000, but weaker than 1:670,000 (confirmed by several observations). It was taken at 1:750,000, corresponding to an output of 0.0008 mgm. per minute for the dog, or 0.00016 mgm. per kilogram per minute. The proportion of serum in the blood was 51 per cent, as determined by the electrical method, and 48 per cent by the haematocrite (after fifty minutes rotation). The epinephrin concentration in the serum of the sixth specimen must, therefore, have been somewhat greater than 1:400,000. It is obvious that with the slow blood flow when the sixth specimen was collected, the calculated output could not possibly equal that for the preceding specimens, unless strophanthin possessed the power, like nicotine, of pushing up the possible maximum concentration far beyond the normal limit.

In reviewing the experiments in which the adrenal blood was assayed on rabbit segments, it is obvious that if strophanthin in the doses employed by us influences the epinephrin output at all, the effect is of a different order of magnitude from that produced by strychnine or from the transient excitation caused by nicotine. And while a marked effect can be elicited by strychnine and nicotine with great constancy, no increase was found in any adrenal blood specimen taken after strophanthin in at least two-thirds of the experiments. In one or two ani-

mals no increase was found in the specimens taken immediately or a few minutes after the administration of the drug, and while it was maintaining a rise of blood pressure, while a remote specimen, collected many minutes thereafter, gave a slightly increased output. In one or two of the animals a slight increase was found in specimens obtained soon after the injection of strophanthin. In no case, either in near or remote specimens, did such an increase as was detected suffice to raise the output beyond the normal range seen in animals under the influence of an ordinary anesthetic alone. The observed increases never amounted to more than 50 to 100 per cent of the initial output before strophanthin, and it is only with rather favorable conditions that changes of much less than 50 per cent in the normal output can be certainly detected with the method employed.

We should hesitate to attribute the occasional small changes observed in the rate of epinephrin output to a stimulating action of strophanthin upon the adrenal secretory mechanism. Whether with a more delicate method, if such should be developed, it would be possible to demonstrate a definite effect of strophanthin on this mechanism, we can, of course, offer no opinion. It appears to us, however, quite likely that a good many drugs, especially such as are known to exert an action upon sympathetic fibers, or on organs supplied by them, may influence the epinephrin output, although not necessarily in a degree detectable or measurable by the best of our present methods.

EXPERIMENTS WITH AUTO-ASSAY BY BLOOD PRESSURE REACTIONS

The experiments with direct collection of adrenal blood and assay of the epinephrin on rabbit segments were controlled by three experiments in which auto-assays by blood pressure reactions were attempted. In one of these, a female cat (314) weighing 2.12 kgm., under urethane, the output of epinephrin was estimated at about 0.0002 mgm. per kilogram per minute by several pocket observations. Strophanthin (0.05 mgm.) was then injected into the jugular vein, the cava pocket closed a few seconds thereafter and adrenal blood collected in the pocket for

two minutes. The effect on the blood pressure of releasing the pocket was about the same as that due to injection (while the pocket was closed off) of 0.5 cc. of a 1:475,000 adrenalin solution, corresponding to an output of about 0.00025 mgm. per kilogram per minute. Another dose of 0.05 mgm. strophanthin was given eight minutes after the first. The strophanthin was put in just after the cava pocket had been closed off. The pocket was kept closed for two minutes and its release produced an effect on the blood pressure somewhat less than that caused by 0.5 cc. of 1:475,000 adrenalin, but decidedly greater than that caused by 0.5 cc. of 1:950,000 adrenalin. The output of epinephrin was, therefore, somewhat less than 0.00025 mgm. per kilogram per minute. Other observations in this experiment showed no increase whatever as compared with the original output before strophanthin. In some observations the output appeared to be decreased rather than increased. A third dose of strophanthin (0.05 mgm.) given thirteen minutes after the second, quickly killed the animal. The adrenals weighed 0.587 gram. The vagi were cut at the beginning of the experiment.

In the second experiment of this group (cat 318) the strophanthin (0.15 mgm. in a 3.3 kgm. male cat) was slowly injected (during ten minutes). The animal was anesthetized with urethane. Release of the cava pocket after being closed for two minutes, before strophanthin, caused a blood pressure reaction much greater than that elicited by injection of 0.25 cc. of 1:130,000 adrenalin, and about the same as that caused by 0.5 cc. of 1:130,000 adrenalin, corresponding to an output of epinephrin of 0.0019 mgm. per minute for the cat, or 0.00057 mgm. per kilogram per minute. A two minute pocket, two to three minutes after the end of the strophanthin injection, gave an effect somewhat greater than that produced by 0.5 cc. of 1:130,000 adrenalin, but much less than that produced by 0.5 cc. of 1:65,000. Another observation, ten minutes after completion of the strophanthin injection, showed that the effect of releasing a pocket after two minutes closure was less than that of injecting 0.5 cc. of 1:100,000 adrenalin and no greater than, if as great as that caused by 0.5 cc. of 1:130,000 adrenalin (confirmed by another observation). A one minute pocket elicited

about the same effect as a one minute pocket before strophanthin. In other words, up to this time no increase in the output had occurred. Release of a two minute pocket, twenty-five minutes after the end of the strophanthin injection caused a decidedly smaller effect than injection of 0.5 cc. of 1:100,000 adrenalin. The effect produced when a pocket which had been closed for three minutes was released was somewhat less than that produced by 0.5 cc. of 1:65,000 epinephrin, but greater than that produced by 0.5 cc. of 1:100,000 adrenalin. Taking the epinephrin collected in the pocket in three minutes as equivalent to 0.5 cc. of 1:80,000 adrenalin, we get 0.002 mgm. per minute as the output for the cat, or 0.0006 mgm. per kilogram per minute, the same as before strophanthin. The adrenals weighed 0.367 gram.

In the third experiment, with blood pressure auto-assay (cat 313), the animal, a female, weighing 3 kgm. was anesthetized with ether. The vagi were cut. Six pocket observations were made before strophanthin was injected, and the output of epinephrin was estimated at about 0.0003 mgm. per kilogram per minute. Strophanthin (0.05 mgm.) was then injected into the jugular vein immediately after the closing off of the pocket. The blood pressure reaction, on releasing it after two minutes closure, seemed qualitatively somewhat increased. This was confirmed by another pocket observation. But it could not be quantitatively shown by comparison with the effects now produced by given doses of adrenalin that there was an increase in the rate of output. A second dose of strophanthin (0.05 mgm.) was then given, thirteen minutes after the first, and the pocket closed in fifteen seconds. No good assay could be made on account of irregularity in the blood pressure curve. But the output was not much, if at all above 0.0003 mgm. per kilogram per minute. The intestinal arteries are usually not tied in such experiments because the preservation of the splanchnic area is favorable to a large epinephrin effect.

The experiments with blood pressure assay leave us accordingly in the same position as those with assay on the rabbit segments, that is, without real evidence of any definite and constant effect of the drug upon the epinephrin output.

SUMMARY

We have been unable to demonstrate any decided and constant effect of strophanthin upon the epinephrin output. Statements in the literature that the drug causes a marked augmentation of the output are based upon the use of inadequate methods.

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THE ACTION OF DRUGS ON THE OUTPUT OF EPINEPHRIN FROM THE ADRENALS

V. CURARA

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Langley and his pupils (1), (2), (3) have shown that curara paralyses many groups of preganglionic fibers by acting upon the peripheral nerve cells. In a recent paper Langley (4), working with cats, gives additional instances in which curara paralyses preganglionic fibers and concludes that it has a more or less paralysing action on all preganglionic nerves. The paralysing action of curara can be overcome by nicotine in sufficient amount. He includes among the fibers paralysed by curara the adrenaline secreting fibers. Although the paper does not appear to contain actual observations in support of this conclusion¹ the assumption is an extremely probable one. It is easy to show that it is correct. Having planned to use curara to paralyse the motor nerves in certain experiments on the epinephrin output, we were compelled to examine the question whether the doses required for this purpose would affect appreciably the adrenal secretory fibers. We found that this was the case and that curara could not be used in the proposed experiments.

Our results show that the epinephrin output in the cat is markedly diminished by the doses necessary to cause and to

¹ In one place Langley says "On the basis of these facts it follows that curara paralyses the adrenaline secreting fibers, and that this paralysis can be overcome by nicotine." The facts referred to are certain observations on the eye, which, so far as we can see, have no direct bearing on the action of curara upon the adrenal nerves, but which may be interpreted as showing that if curara paralyses the adrenaline secreting fibers, the paralysis can be overcome by nicotine.

maintain complete paralysis of the skeletal musculature even for a relatively short time and the diminution may be still quite definite when stimulation of the cardioinhibitory fibers, if they have been paralysed by the dose of curara, is again effective. We did not attempt to determine exactly the relative susceptibility of the epinephrin-secreting fibers and of the other groups of autonomic fibers as this had no bearing on the subject of our investigation. But since the cardio-inhibitory fibers are the most easily paralysed by curara of all the groups, according to Langley (4), we do not think that the adrenaline secreting fibers are correctly placed by him among the fibers which are paralysed with the greatest difficulty. His paper does not show on what evidence this classification of the adrenal fibers is based.

Our experiments were made on cats and with the technique described in previous papers (5), the epinephrin in adrenal vein blood collected from a pocket of the vena cava being assayed on rabbit intestine (and uterus) segments. The animals were anesthetized with urethane. The smallest dose of curara employed was 0.25 cc. of a 1 per cent solution per kilogram (0.75 cc. in a 3 kgm. cat), and the largest 0.5 cc. of the same solution per kilogram. The real dose would be somewhat greater as the abdominal aorta was tied at the bifurcation when the cava pocket was made. These doses caused complete paralysis of the skeletal muscles as shown by stimulation of the brachial nerve. The respiration ceased, but at the end of the short experiment spontaneous respiratory movements were observed after stopping the artificial respiration, and stimulation of the brachial nerve caused some contraction. Paralysis of the cardio-inhibitory fibers could be demonstrated but it was not lasting, and samples of adrenal vein blood collected after distinct recovery of the conductivity in the cardio-inhibitory path showed still a marked diminution of the epinephrin output.

A few samples of tracings used in the assay and protocols of the experiments follow.

Condensed protocol. Cat 378, male, weight, 3.07 kgm. Anesthetized with urethane

- 10.30 a.m. Cannulae inserted in trachea, external jugular vein and carotid artery; left vagus ligated and cut.
- 10.55 a.m. Cava pocket completed. Collected adrenal blood.
- 10.55 a.m. First specimen, 1.6 gram in 30 seconds (3.2 grams per minute). 10.55½ a.m. Second specimen, 5.35 grams in 120 seconds (2.7 grams per minute).
- 11.05 to 11.15 a.m. Blood pressure 120 mm. mercury. In this interval vagus stimulated 3 times. Stimulation caused marked fall of blood pressure and slowing of heart.
- 11.15 a.m. Started artificial respiration and began injection of curara.
- 11.15½ a.m. End of intravenous injection of 0.75 cc. of curara (1 per cent in physiological salt solution). Blood pressure 68 mm., falling during the next minute to 58 mm. mercury.
- 11.17 a.m. Cessation of spontaneous respiration. Vagus stimulation had no effect on blood pressure or heart rate.
- 11.18 a.m. Third adrenal specimen, 0.75 gram in 30 seconds (1.5 gram per minute).
- 11.18½ a.m. Fourth adrenal specimen, 4.7 grams in 210 seconds (1.34 gram per minute).
- 11.22 a.m. Blood pressure 78 mm. mercury.
- 11.22 to 11.26 a.m. Vagus stimulated 3 times. Caused a moderate fall of blood pressure and with stronger stimulation cessation of the heart beat.
- 11.26 a.m. Blood pressure 53 mm. mercury.
- 11.35 a.m. Stimulation of peripheral end of brachial nerve caused contraction of muscles. Spontaneous respiration not yet returned.
- 11.36 a.m. Fifth adrenal specimen, preliminary, collected for 60 seconds.
- 11.37 a.m. Sixth adrenal specimen, 1.7 gram in 360 seconds (0.3 gram per minute).
- 11.43 a.m. Palpation of abdominal aorta during vagus stimulation showed slowing and weakening of heart beat. Obtained indifferent blood from the cava (after tying off the adrenal veins). During the collection of this blood gasping movements were observed. Exposed heart and stimulated vagus; the auricles almost stopped and the ventricles were slowed. Combined weight of adrenals 0.518 gram.

The second adrenal blood specimen, collected before injection of curara, was shown to be much stronger than 1:6,600,000 adrenalin, decidedly stronger than 1:5,300,000. (fig. 1, observations 10, 14 and 16, confirmed by other observations not reproduced), much weaker than 1:2,700,000 (observation not reproduced), somewhat weaker than 1:4,000,000 (fig. 1, observation 12). It was finally assayed at 1:4,500,000, corresponding to an output of 0.0006 mgm. per minute for the cat, or 0.0002 mgm. per kilogram of body weight per minute, not far from the average normal output under our experimental conditions.

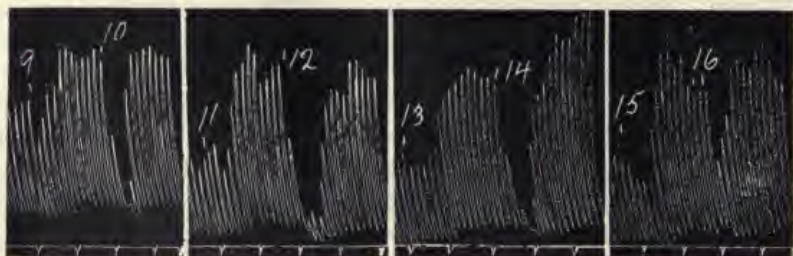


FIG. 1. INTESTINE TRACINGS. BLOODS FROM CAT 378

At 9, 11, 13, and 15 Ringer was replaced by jugular blood, and this at 10 by jugular blood to which was added adrenalin to make a concentration of 1:5,300,000; at 12 by jugular blood to which was added adrenalin to make a concentration of 1:4,000,000; at 14 by the second adrenal blood specimen (collected before injection of curara); at 16 by jugular blood to which was added adrenalin to make a concentration of 1:6,600,000. All the bloods were diluted with three volumes Ringer, the adrenalin bloods after adding the adrenalin. (Reduced to two-thirds.)

The fourth adrenal specimen, collected $3\frac{1}{2}$ minutes after completion of the curara injection and about $1\frac{1}{2}$ minutes after paralysis of respiration was seen to be complete, was weaker than 1:5,300,000 adrenalin, decidedly stronger than 1:8,000,000 (fig. 2, observations 22 to 26, confirmed by observations 28 to 36). Two duplicate sets of observations are reproduced in figure 2 to illustrate a point in the technique of these intestine segment assays. The segment was beating more strongly when the lower row of tracings was taken, but it will be noted that this does not at all disturb the relative magnitude of the inhibitory reactions. Two separate observations (not reproduced)

indicate that the 4th specimen was slightly weaker than 1:6,600,000. It was taken at 1:6,700,000, corresponding to an output of 0.0002 mgm. per minute for the cat, or 0.000065 mgm. per kilogram per minute, about one-third of the original output.

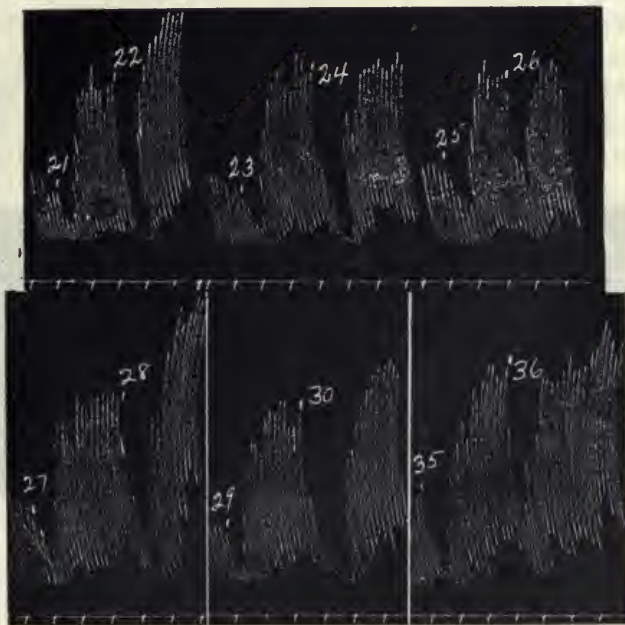


FIG. 2. INTESTINE TRACINGS. BLOODS FROM CAT 378

At 21, 23, 25, 27, 29, and 35 Ringer was replaced by indifferent (venous) blood (collected after injection of curara), and this at 22 and 28 by the fourth adrenal blood specimen (collected 3 minutes after injection of curara); at 24 and 30 by the indifferent blood to which was added adrenalin to make a concentration of 1: 5,300,000; at 26 and 36 by the indifferent blood to which was added adrenalin to make a concentration of 1: 8,000,000. All the bloods were diluted with three volumes Ringer, the adrenalin bloods after adding the adrenalin. (Reduced to one half.)

The sixth adrenal blood specimen, procured 22½ minutes after the injection of curara, was much weaker than 1: 2,700,000 (fig. 3, observations 46 and 48, confirmed by another set of observations not reproduced), and stronger than 1: 4,000,000 (fig. 3, observation 44). It was assayed at 1: 3,400,000, corre-

sponding to an output of 0.00009 mgm. per minute for the animal, or 0.00003 mgm. per kilogram per minute, not one-sixth of the original output. Although the blood flow at the time of collection of the sixth specimen was greatly reduced this reduction was not accompanied by a correspondingly increased concentration, as would be the case in an animal under urethane alone. The concentration is far below the possible maximum. There is no question then that curara produced a marked degree of paralysis of the epinephrin secretory path, which had not yet reached its maximum at a time when paralysis of the skeletal

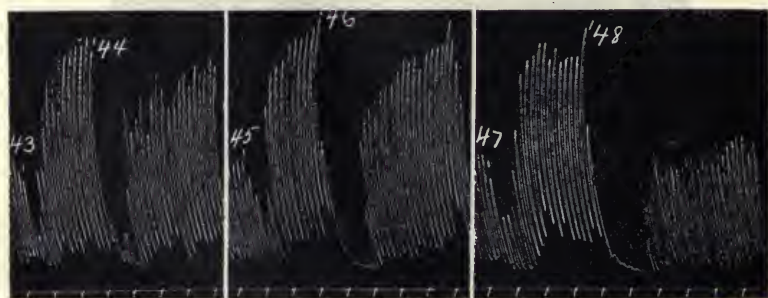


FIG. 3. INTESTINE TRACINGS. BLOODS FROM CAT 378

At 43, 45 and 47 Ringer was replaced by indifferent (venous) blood (collected after injection of curara) and this at 44 by the indifferent blood to which was added adrenalin to make a concentration of 1: 5,000,000; at 46 by the sixth adrenal blood specimen (collected 22 minutes after injection of curara); at 48 by the indifferent blood to which was added adrenalin to make a concentration of 1: 2,700,000. All the bloods were diluted with three volumes Ringer, the adrenalin bloods after adding the adrenalin. (Reduced to one-half.)

muscles was complete and at a time when the paralysis of the cardio-inhibitory fibers was wearing off.

The question might be raised whether the low blood pressure during collection of the sixth specimen might not be a factor in continuing and deepening the paralysis of the adrenal secretory fibers. We have abundant evidence that in an animal under urethane alone such an effect is not produced even when the blood flow through the adrenal and the blood pressure are still smaller than in this case. But it may be asked whether the paralysing action of curara might not be intensified by the

unfavorable influence of the low blood pressure. We have no evidence as to this and can only point out that the cardio-inhibitory peripheral cells, which are equally exposed to adverse effects of the low blood pressure, seem to have been recovering at a time when no recovery could be noted in the epinephrin secretion. It ought to be remarked that we were studying the influence of curara upon the spontaneous liberation of epinephrin, and a comparison of the effect produced by a given dose of the drug upon the conductivity to impulses originated by artificial stimulation of the preganglionic fibers in the one case and upon the conductivity to impulses originating without artificial stimulation in the central mechanism in the other case, may not be formally correct. The matter is of no importance for our purpose, which was accomplished as soon as it became evident that the epinephrin secretory fibers were among those most easily affected by curara and not among those most refractory to the drug. For this reason also it was of no importance to us to determine the relative ease of total paralysis of the adrenal path, although our experience with nicotine makes it probable that a moderate increase in the dose would bring us to the point where the epinephrin output would be undetectable with the test objects employed. Also it did not seem worth while to verify the ultimate restitution of conductivity with the smaller doses, as in the case of nicotine, since it could be assumed that this would occur, if the circulatory conditions remained favorable.

In the next experiment (cat 377) the dose per kilogram of bodyweight was somewhat increased, to about 0.4 cc. of the 1 per cent solution. Respiratory movements ceased promptly and collection of adrenal blood was begun within 20 seconds of the end of injection of curara, in order to see whether any transient stimulating action on the epinephrin secretion could be detected, as with nicotine.

Condensed protocol. Cat 377, female, weight, 2.7 kgm.

- 9.20 a.m. Anesthetized with ether. Inserted tracheal and jugular cannulae. Obtained indifferent blood from external jugular vein.
- 10.00 a.m. Cava pocket completed. Started artificial respiration. Collected adrenal blood.
- 10.00 a.m. First specimen, 4.6 grams in 30 seconds (9.2 grams per minute).
- 10.00½ a.m. Second specimen, 9.95 grams in 90 seconds (6.6 grams per minute).
- 10.05½ a.m. End of intravenous injection of 1.0 cc. curara solution (1 per cent in physiological salt solution).
- 10.06 a.m. Third adrenal specimen, 4.05 grams in 30 seconds (8.1 grams per minute).
- 10.06½ a.m. Fourth adrenal specimen, 7.5 grams in 90 seconds (5.0 grams per minute).
- 10.08 a.m. Fifth adrenal specimen, 5.8 grams in 120 seconds (2.9 grams per minute).
- 10.18 a.m. Cat died. Combined weight of adrenals 0.367 gram.

The assay on rabbit intestine segments showed that the second adrenal blood specimen, collected before injection of curara, was stronger than 1:8,600,000 adrenalin, stronger than 1:7,000,000, decidedly weaker than 1:4,300,000, somewhat weaker than 1:5,700,000. Taking the concentration at 1:6,000,000 we get 0.0011 mgm. epinephrin per minute for the cat, or 0.0004 mgm. per kilogram per minute. This output is within the normal range, though higher than the average. The 5th specimen, collection of which was begun 2½ minutes after completion of the curara injection, was much weaker than 1:5,700,000 adrenalin, and certainly no stronger than 1:14,000,000, probably somewhat weaker. Since the blood flow during collection of the 5th specimen was less than half as great as for the second, this alone shows that the output was much reduced by the curara. Taking the fifth specimen at 1:14,000,000 epinephrin, we get 0.0002 mgm. per minute for the cat, or 0.000075 mgm. per kilogram per minute, not one-fifth of the original output before curara. The fourth specimen, collection of which

was begun less than a minute after the curara injection had been completed, had a concentration of epinephrin not very different from that in the 5th specimen, if anything somewhat greater, but far less than in the second. It was shown to be much weaker than 1:5,700,000 and probably somewhat weaker than 1:14,000,000. Since the blood flow for the fourth was 70 per cent greater than for the fifth specimen, the curara paralysis of the epinephrin secretion, although quite evident at the time of collection of the fourth specimen, the output being reduced to one-third to one-fourth of the original output, had not reached its maximum and was still developing.

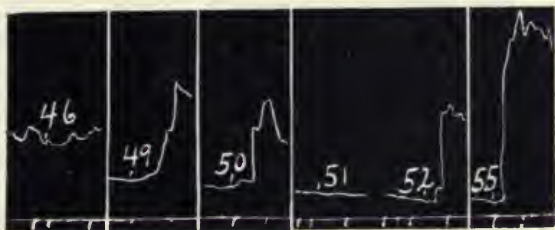


FIG. 4. UTERUS TRACINGS. BLOODS FROM CAT 377

At 46 Ringer was replaced by indifferent (venous) blood (collected after injection of curara); at 49 by the fifth adrenal blood specimen (collected 2½ minutes after injection of curara); at 50 by the third adrenal blood specimen (collected immediately after injection of curara); at 51 by jugular blood (collected before injection of curara); at 52 by the fourth adrenal blood specimen (collected 1 minute after injection of curara); at 55 by the second adrenal blood specimen (collected before injection of curara). All the bloods were diluted with three volumes Ringer. (Reduced to two-thirds).

As so many intestine tracings have been reproduced to illustrate the assay on the bloods from cat 378, only a few uterus tracings from bloods of cat 377 are given (fig. 4). They show qualitatively that the second specimen is much stronger than the other adrenal bloods and that these cause a decidedly greater increase of tone than indifferent blood. It was also confirmed by the uterus that the second specimen did not differ much from a 1:5,700,000 concentration of adrenalin in the indifferent blood.

The largest dose of curara given was to cat 376, about double the dose per kilogram used for cat 378.

Condensed protocol. Cat 376, female, weight, 2.16 kgm. Anaesthetized with urethane

- 9.40 a.m. Inserted tracheal and jugular cannulae and obtained indifferent blood from external jugular vein.
- 10.08 a.m. Cava pocket completed. Collected adrenal blood.
- 10.08 a.m. First specimen, 0.5 gram in 30 seconds (1 gram per minute).
- 10.08½ a.m. Second specimen, 4.15 grams in 240 seconds (1.04 gram per minute).
- 10.18 a.m. End of intravenous injection of 1.0 cc. of curara solution (1 per cent in physiological salt solution).
- 10.20 a.m. Cessation of spontaneous respiration. Started artificial respiration.
- 10.20 a.m. Third adrenal specimen, 0.45 gram in 45 seconds (0.6 gram per minute).
- 10.20¾ a.m. Fourth adrenal specimen, 3.55 grams in 360 seconds (0.6 gram per minute).
- 10.30 a.m. Stimulation of peripheral end of brachial nerve caused contraction of muscles (toe movements).
- 10.40 a.m. Fifth adrenal specimen, preliminary, collected for 60 seconds.
- 10.41 a.m. Sixth adrenal specimen, 1.9 gram in 360 seconds (0.3 gram per minute).
- Obtained another specimen of indifferent blood from the cava (after tying off the adrenal veins). Respiratory gasps during collection of this blood. Combined weight of adrenals 0.472 gram.

The second adrenal specimen, procured before injection of curara, was found to be decidedly weaker than 1:2,850,000 adrenalin, weaker than 1:4,300,000 (confirmed by several observations), much the same as 1:5,700,000 (confirmed by 4 sets of observations). Taking it at 1:5,700,000 we get 0.00018 mgm. per minute for the cat, or 0.00008 mgm. per kilogram per minute, considerably less than the average output. The fourth specimen, collection of which was begun 2¾ minutes after completion of the curara injection, was much weaker than 1:10,000,000, weaker than 1:11,400,000, no stronger than 1:14,000,000. Taking it at 1:14,000,000 we get an epinephrin output of 0.00004

mgm. per minute for the cat, or 0.00002 mgm. per kilogram per minute, only one-fourth of the original output before curara. The sixth adrenal specimen, collection of which was begun 23 minutes after injection of curara, was found to be much weaker than 1:2,850,000, stronger than 1:7,000,000, somewhat weaker than 1:4,300,000. Taking it at 1:5,000,000 we get an output of 0.00006 mgm. per minute for the cat, or 0.00003 mgm. per kilogram per minute. With the small blood flow a much greater concentration of epinephrin would have been expected in the sixth specimen, in the absence of curara. The paralysis was, therefore, still very evident at this time. The assay was not good enough to permit the conclusion that the output had recovered somewhat at the time of collection of the sixth specimen as compared with the fourth.

The ease with which curara depresses the epinephrin output raises the question whether the transient diminution in the output sometimes observed by us after strychnine (5), preceding the marked and long-lasting augmentation caused by that drug, may not be due to a transient paralysis of peripheral nerve cells on the efferent secretory path, since Langley has shown that strychnine, like curara, has a very general paralysing action on such nerve cells. However, the doses of strychnine employed by Langley to obtain paralysis of other groups of pre-ganglionic fibers are enormous in comparison with the doses whose effect upon the epinephrin secretion we have studied.

SUMMARY

1. Curara in doses sufficient to paralyse the skeletal muscles in the cat markedly depresses the output of epinephrin from the adrenals. The depression begins promptly and may be still well marked when paralysis of the muscles has begun to wear off. While no attempt was made to compare exactly the doses required to paralyse the epinephrin-secretory fibers and the cardio-inhibitory fibers, a marked diminution in the epinephrin output was observed in samples of blood collected from

the adrenals at a time when stimulation of the vagus caused inhibition of the heart.

2. In general, curara should not be employed in experiments on the epinephrin output.

REFERENCES

- (1) LANGLEY AND DICKINSON: Journ. Physiol., 1890, xi, 509.
- (2) LANGLEY AND ANDERSON: Journ. Physiol., 1892, xiii, 460.
- (3) LANGLEY: Journ. Physiol., 1911, xliii, 125.
- (4) LANGLEY: Journ. Physiol., 1918, lii, 247.
- (5) STEWART AND ROGOFF: Journ. Pharm. and Exp. Therap., 1919, xiii, 95.

ERRATA, VOL. XVI, NO. 2, SEPTEMBER

Page 71. Line 23 from bottom, for "secretory" read "salivary."

Page 72, before "slower" insert "generally;" after "rabbit" insert "when blood is collected in this way."

Page 74, line 14 from bottom, for "find" read "found;" line 13 from bottom, for "incredibly" read "extraordinarily."

Page 107, line 4, delete "Whatever action;" line 12, for "in" read "with."

#19.
partis juice

THE ACTION OF DRUGS ON THE OUTPUT OF EPINEPHRIN FROM THE ADRENALS VI. ATROPINE; PILOCARPINE

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The well-known peripheral action of atropine and of pilocarpine on structures innervated by parasympathetic nerves, especially the heart and secretory glands, and on the secretion of sweat, which is under the influence of true sympathetic nerves, suggests that these drugs might exert an action on the secretion of epinephrin from the adrenal glands. There is, at present, no well established information bearing on this question, based upon experimental evidence obtained with methods that are sufficiently sensitive and capable of yielding quantitative results.

Meltzer and Auer (1) observed that the enucleated frog's bulbus reacted to small amounts of adrenalin by dilatation and suggested that this might prove to be a better reagent than the blood-pressure to demonstrate the efficiency of a suprarenal preparation. Ehrmann (2), employing this reaction, concluded that in rabbits and cats there is no noteworthy effect produced by atropine or pilocarpine on the epinephrin secretion from the adrenals. He obtained blood through a cannula in the cava just below the entrance of the renal veins. The renal vessels were tied at the hilus, the abdominal aorta and small veins entering the cava above the cannula were tied and a ligature on the cava just beneath the liver completed a pouch which permitted only the blood from the adrenal and first lumbar veins to flow through the cannula. Serum was obtained from this blood by centrifuging and diluted five, ten and twenty times with physiological salt solution, and applied to the frog bulbus. Systemic blood-serum, in like dilutions, was used as a control. Ehrmann states that the concentration

of adrenalin in the adrenal vein blood of the rabbit is between 1:1,000,000 and 1:10,000,000 and in the cat it is considerably less, but he does not take account of the differences in the rate of blood-flow through the adrenals. The greater concentration of epinephrin in the adrenal blood of the rabbit than in that of the cat can be explained by the slower flow through the adrenals in the rabbit. Differences in the amount of dilution of the adrenal vein blood by the blood from the first lumbar veins would also contribute to the differences in the epinephrin concentrations in the bloods collected by Ehrmann. His experiments yield no quantitative information on the rate at which epinephrin was being secreted from the adrenals.

The enucleated frog's eye as a test for epinephrin was carefully studied and critically considered by Schultz (3). He found that the degree of mydriasis is not proportional to the strength of the adrenalin solution applied. At 23°C. solutions which contain less than 1:625,000 adrenalin yield a reaction so small as to be of uncertain origin. Schultz also makes the pertinent suggestion that substances in sera, other than adrenalin may materially modify the reaction. A test object that cannot be relied upon to detect, with certainty, concentrations of epinephrin below 1:625,000 can, at best, be of use only for qualitative reactions. Such a concentration of epinephrin, under ordinary experimental conditions, is very nearly the maximal found in adrenal vein blood collected with the slowest blood-flows, in the absence of splanchnic stimulation, massage of the glands or administration of certain drugs (nicotine), which are capable of augmenting the output of epinephrin to yield a concentration above the ordinary possible maximum.

Popielski (4) studied the effect, on the blood-pressure, of releasing the aorta after clamping it off in the thorax for three to seven minutes. Although he made no quantitative observations, he found no alteration in the degree of the reaction after administering atropine. The epinephrin factor in the blood-pressure reaction thus obtained is contributed by the entrance into the circulation of the epinephrin which is secreted into the adrenal capillaries and veins during the period of occlusion of the aorta and is washed into the blood stream when the circulation through the glands is re-established. We have found, in similar experiments, that short periods of anemia, thus produced, do not materially interfere with the secretion of epinephrin from the adrenals, and that, after release of the aorta, the epinephrin secreted under the influence of splanchnic stimulation while the circulation through the adrenals was interfered with, is washed into the circulation

and produces the characteristic reactions on the pupil and nictitating membrane of the eye previously rendered sensitive to epinephrin by removal of the superior cervical ganglion (5).

Biedl (6) found no diminution in the epinephrin secretion from the adrenals, after administration of atropine, on stimulation of the splanchnic nerve. Tchekoksaroff (7), employing the same method as Biedl, confirmed this observation and, further observed that no demonstrable effect on the epinephrin secretion was produced by administration of pilocarpine. His experiments were performed on dogs. The abdomen was opened in the midline from the xiphoid to the symphysis and a transverse incision then made on the left side of the abdomen parallel with and near the costal margin and the flaps laid open. The abdominal viscera, covered with warm towels, were laid over to the right side, a cannula inserted into the left lumbar vein and the left adrenal vein tied at its entrance into the cava, permitting only the blood coming from the left adrenal to flow through the cannula. The rate of blood-flow through the adrenal, at the time of collection of the blood, was taken account of by counting the number of drops that flowed from the cannula in a given time. The blood, after defibrination, was then injected, in 10 cc. quantities, into the jugular vein of another dog weighing 5 to 8 kgm., and the effect on the blood-pressure and heart action observed. Tchekoksaroff found that adrenal vein blood collected during stimulation of the peripheral end of the splanchnic nerve, after administration of 10 mgm. of atropine, produced fully as great an effect on the blood-pressure and heart action, when introduced into another dog, as the blood collected before atropine was injected. Pilocarpine, administered in doses of 5 to 10 mgm, after section of the splanchnic, produced a copious flow of saliva and pancreatic juice, but the adrenal vein blood collected after the injection of pilocarpine caused no greater effect, when introduced into the circulation of another dog than the blood collected before the drug was injected, or than the introduction of an equal quantity of arterial blood. In the experiments performed by Tchekoksaroff the splanchnic nerve was sectioned and only the peripheral effects of these drugs could have been observed. The interference with the nerve supply to the adrenal in some of his experiments apparently had so diminished the epinephrin output from the adrenal that the blood from the gland gave no greater reaction than arterial blood. The method of assay of the adrenal vein blood, employed by him, although not so sensitive as the use of segments of rabbit's intestine and uterus, is capable of yielding quantitative data.

Dale and Laidlaw (8) investigated the action of pilocarpine on the rate of output of epinephrin from the adrenals in cats. They employed, as a test object for epinephrin, the isolated non-pregnant cat's uterus and collected blood from the adrenals through a cannula in a pouch of the cava. In one experiment they used a loop of rabbit's intestine as the test object. The animal was pithed, the abdomen opened and the whole alimentary canal, from the cardiac orifice of the stomach to the lower part of the rectum was removed, after ligation of all the vessels. The portion of cava including both adrenal veins was isolated and a cannula inserted in the lower end for collection of the adrenal vein blood. Fifty milligrams of hirudin was injected into the jugular vein of the cat. In some of the experiments small tributary veins were left unligatured and the adrenal vein blood was diluted considerably. They performed five experiments, in only one of which was there an assay made of the amount of epinephrin in the adrenal vein blood. In experiments 1, 3 and 4 quoted by Dale and Laidlaw they found that pilocarpine had doubled the output of epinephrin per unit of time. Experiment 5 gave the same result although the uterus was not sensitive, while experiment 2 showed no change. In the fourth experiment the concentration of epinephrin in the adrenal blood collected after injection of pilocarpine (blood flow 5 cc. in 83 seconds) was assayed at 1:200,000, giving an output of 0.01 mgm. in 34 seconds. This corresponds to about twenty times the average normal output usually found by us in cats under urethane or ether. Dale and Laidlaw did not assay the epinephrin content of the adrenal vein blood collected before injection of pilocarpine in this experiment, but since they find qualitatively that the output after pilocarpine was doubled, the initial output must have been incredibly high, i.e., about ten times the rate found by us using rabbit's intestine and uterus segments for the assays.

The observations made by Dale and Laidlaw on the eye, after removal of the superior cervical ganglion, could not have yielded quantitative information on the rate of output of epinephrin from the adrenals. Obviously, even qualitative observations on this test object must be cautiously interpreted when studying the effect of a drug like pilocarpine on the adrenal secretion.

Our experiments on the influence of atropine and of pilocarpine on the rate of output of epinephrin from the adrenals were made on cats. The method of obtaining adrenal vein blood has been sufficiently described in previous papers (9). The adrenal speci-

mens were assayed on rabbit's intestine and uterus segments. In all of these experiments, except one with pilocarpine (cat 466), the drugs were introduced through a cannula in the external jugular vein. The actual dose administered would, in every case, be somewhat greater than that mentioned, as the abdominal aorta was tied off near the bifurcation when the cava pocket was made. In general, it may be stated that the results of our experiments lead us to the same conclusions arrived at by other investigators. No significant alteration in the rate of output of epinephrin from the adrenals is caused by either of these drugs. Certainly, any effect that might be demonstrated (if any genuine effect exists) is not comparable to the pronounced influence on the epinephrin output demonstrated by the action of strychnine (9), nicotine (10), and curara (11). Condensed protocols of some of the experiments and samples of tracings used in the assays are given.

THE INFLUENCE OF ATROPINE ON THE EPINEPHRIN OUTPUT

The smallest dose of atropine employed was 0.15 mgm. per kilogram of body weight. This amount always produced marked dilation of the pupils and paralysis of the vagus endings in the heart, but no diminution in the rate of output of epinephrin from the adrenals. Indeed, the assays indicate that there may have been a small augmentation of the epinephrin output. Tchekboksaroff suggested that splanchnic stimulation, after administration of atropine, seemed to produce a greater output of epinephrin than stimulation before atropine was injected.

Condensed protocol. Cat 459; female; weight 2.92 kgm.

Under ether inserted cannulae into trachea, external jugular vein and carotid artery. Obtained indifferent (jugular) blood.

9.50 a.m. Cava pocket completed;¹ collected adrenal blood.

¹ In all of the experiments the "cava pocket" was made by tying the renal arteries and veins at the hilus (another ligature was tied around each renal vein just before it enters the cava and the lumbar veins tied just before they cross the adrenals), the abdominal aorta just above the bifurcation, and the small tributaries entering the segment of cava which constitutes the pocket. The cannula was inserted into the lower end of the segment of cava and the pocket completed, just before beginning of collection of a specimen, by placing a clip on the cava just below the liver.

- 9.50½ a.m. First specimen, 0.7 gram in 30 seconds (1.4 grams per minute).
- 9.51 a.m. Second specimen, 3.6 grams in 3 minutes (1.2 grams per minute).
- 10.04½ a.m. End of intravenous injection of 0.45 mgm. atropine sulphate; blood pressure 90 mm. of mercury.
- 10.05 a.m. Third specimen, 2.1 grams in 1 minute.
- 10.06 a.m. Fourth specimen, 4.35 grams in 2½ minutes (1.74 grams per minute); blood pressure 92 mm. of mercury.
- 10.20 a.m. Stimulation of peripheral end of vagus caused no effect on heart rate or blood pressure.
- 10.33½ a.m. Fifth specimen, 0.6 gram in 30 seconds (1.2 grams per minute).
- 10.34 a.m. Sixth specimen, 3.45 grams in 4 minutes (0.86 gram per minute); blood pressure 70 mm. of mercury at beginning of collection, falling to 62 mm. of mercury at end.

Obtained another specimen of indifferent blood. Combined weight of adrenals 0.302 gram.

The assay on rabbit's intestine segments showed that the second adrenal specimen, collected before injection of atropine, was somewhat stronger than 1:2,000,000 adrenalin, much weaker than 1:900,000, stronger than 1:2,660,000 (fig. 1, observations 14 and 16) and weaker than 1:1,330,000 (fig. 1, observation 18). It was finally assayed at 1:1,600,000, corresponding to an output of 0.00075 mgm. per minute for the cat, or 0.00026 mgm. per kilogram of body weight. Three other sets of observations corroborated this assay.

The third specimen, collected immediately after injection of atropine, and the fourth specimen, collected 1½ minutes after injection of atropine, had the same epinephrin concentration, much greater than 1:2,660,000, weaker than 1:800,000 and very well matched by 1:1,330,000 (fig. 2, observations 26 to 30). After confirmation by other observations, they were taken at 1:1,330,000, corresponding to an output, in the third specimen, of 0.0015 mgm. per minute for the cat, or 0.0005 mgm. per kilogram, and in the fourth specimen, 0.0013 mgm. per minute for the cat, or 0.00045 mgm. per kilogram of body weight.

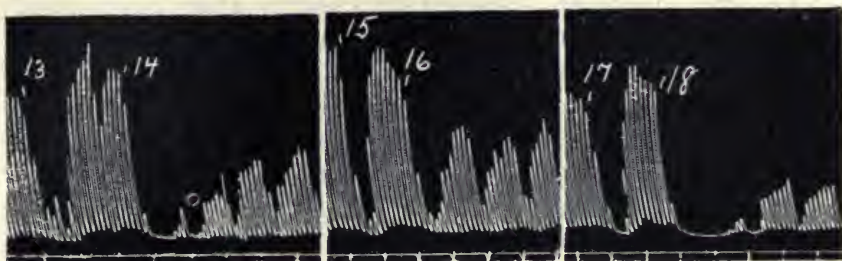


FIG. 1. INTESTINE TRACINGS. BLOOD FROM CAT 459

At 13, 15 and 17, Ringer was replaced by jugular blood and this at 14 by the second adrenal specimen (collected before intravenous injection of atropine) diluted with 4 volumes of jugular blood; at 16 by jugular blood to which was added adrenalin to make a concentration of 1:13,300,000; at 18 by jugular blood to which was added adrenalin to make a concentration of 1:6,600,000. All the bloods were diluted with 5 volumes Ringer (the adrenal blood after dilution with jugular blood and the adrenalin bloods after adding the adrenalin). Reduced to two-thirds.

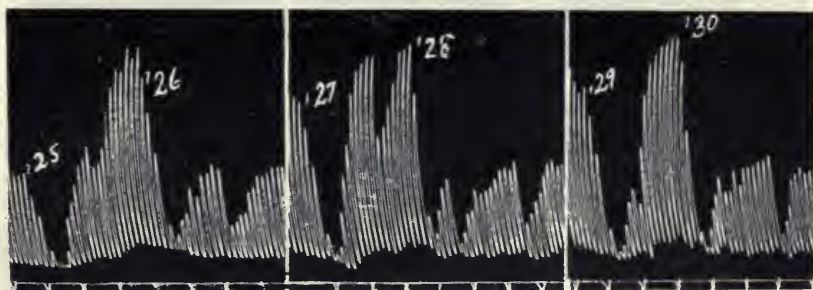


FIG. 2. INTESTINE TRACINGS. BLOODS FROM CAT 459

At 25, 27, and 29, Ringer was replaced by indifferent blood (collected after intravenous injection of atropine) and this at 26 by the fourth adrenal specimen (collected 1½ minutes after intravenous injection of atropine) diluted with 4 volumes of indifferent blood; at 28 by indifferent blood to which was added adrenalin to make a concentration of 1:6,650,000; at 30 by the third adrenal specimen (collected immediately after injection of atropine) diluted with 4 volumes indifferent blood. All the bloods were diluted with 3 volumes Ringer (the adrenalin blood after adding the adrenalin and the adrenal bloods after dilution with the indifferent blood). Reduced to two-thirds.

The sixth specimen, obtained 30 minutes after injection of atropine, was weaker than 1:530,000 (fig. 3, observations 40 and 42) and stronger than 1:800,000 (fig. 3, observation 44). This was confirmed on two other sets of observations. It was assayed at 1:700,000, corresponding to an output of 0.0012 mgm. per minute for the cat, or 0.00041 mgm. per kilogram of body weight. In the next experiment (cat 462) the initial output of epinephrin from the adrenals, before injection of atropine, was low. The animal was pregnant.

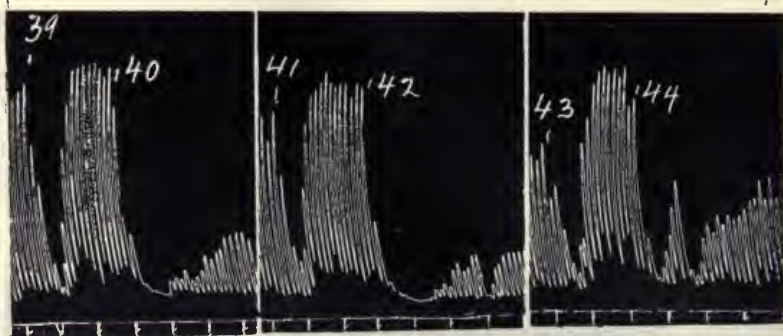


FIG. 3. INTESTINE TRACINGS. BLOODS FROM CAT 459

At 39, 41 and 43, Ringer was replaced by indifferent blood (collected after intravenous injection of atropine) and this at 40 by the sixth adrenal specimen (collected 30 minutes after injection of atropine) diluted with 4 volumes indifferent blood; at 42 by indifferent blood to which was added adrenalin to make a concentration of 1:2,700,000; at 44 by indifferent blood to which was added adrenalin to make a concentration of 1:4,000,000. All the bloods were diluted with 3 volumes Ringer (the adrenal blood after dilution with indifferent blood and the adrenalin bloods after adding the adrenalin). Reduced to three-fourths.

Condensed protocol. Cat 462; female (pregnant); weight 2.55 kgm.

Under ether inserted cannulae into trachea, external jugular vein and carotid artery. Obtained indifferent (jugular) blood.

9.45 a.m. Cava pocket completed; collected adrenal blood.

9.50 a.m. First specimen, 1.1 grams in 30 seconds (2.2 grams per minute).

9.50½ a.m. Second specimen, 3.65 grams in 2 minutes (1.8 grams per minute); blood pressure 82 mm. of mercury.

- 10.01 a.m. End of intravenous injection of 0.4 mgm. atropine sulphate; blood pressure 91 mm. of mercury.
- 10.01½ a.m. Third specimen, 1 gram in 30 seconds (2 grams per minute).
- 10.02 a.m. Fourth specimen, 3.85 grams in 2½ minutes (1.54 grams per minute); blood pressure 74 mm. of mercury.
- 10.12 a.m. Stimulation of peripheral end of vagus caused no effect on heart rate or blood pressure (61 mm. of mercury).
- 10.20½ a.m. Fifth specimen, 0.75 gram in 30 seconds (1.5 grams per minute).
- 10.21 a.m. Sixth specimen, 3.4 grams in 3 minutes (1.13 grams per minute); blood pressure 56 mm. of mercury.

Another specimen of indifferent blood was obtained. Combined weight of adrenals 0.310 gram.

The second specimen, obtained before injection of atropine, was decidedly weaker than 1:4,300,000 adrenalin, somewhat weaker than 1:8,600,000, much stronger than 1:14,000,000, and somewhat stronger than 1:10,700,000. It was finally assayed at 1:9,000,000, corresponding to an output of 0.0002 mgm. per minute for the cat, or 0.00008 mgm. per kilogram. The fourth specimen, collected 1½ minutes after injection of atropine, was decidedly stronger than 1:7,000,000, and weaker than 1:2,850,000. It was assayed at 1:4,300,000, corresponding to an output of 0.00034 mgm. per minute for the cat, or 0.00013 mgm. per kilogram. The sixth specimen, obtained 20 minutes after administration of atropine, was decidedly stronger than 1:7,000,000 adrenaline, somewhat stronger than 1:4,300,000, and weaker than 1:2,850,000. It was finally assayed at 1:3,500,000, corresponding to an output of 0.0003 mgm. per minute for the cat, or 0.00012 mgm. per kilogram.

In the above experiments (cats 459 and 462) the dose of atropine administered was 0.15 mgm. per kilogram of body weight. The results obtained in both experiments indicate an apparent increase in the rate of epinephrin output from the adrenals, of approximately 50 per cent.

In the next experiment (cat 458) the dose of atropine was 0.9 mgm. per kilogram of body weight. A few of the tracings used in the assay are given.

Condensed protocol. Cat 458; female (pregnant); weight 2.21 kgm.

Under ether inserted cannulae into trachea, external jugular vein and carotid artery. Obtained indifferent (jugular) blood.

10.10 a.m. Cava pocket completed; collected adrenal blood.

10.12½ a.m. First specimen, 0.65 gram in 30 seconds (1.3 grams per minute).

10.13 a.m. Second specimen, 3.7 grams in 2 minutes (1.85 grams per minute); blood pressure 118 mm. of mercury.

10.18½ a.m. End of intravenous injection of 2 mgm. atropine sulphate; blood pressure 110 mm. of mercury.

10.19 a.m. Third specimen, 2.1 grams in 1 minute.

10.20 a.m. Fourth specimen, 3 grams in 2 minutes (1.5 grams per minute); blood pressure 62 mm. of mercury.

10.35 a.m. Stimulation of peripheral end of vagus caused no effect on heart rate or blood pressure.

10.37 a.m. Fifth specimen, 1.5 grams in 1 minute.

10.38 a.m. Sixth specimen, 4.95 grams in 3 minutes (1.65 grams per minute); blood pressure 63 mm. of mercury.

Obtained another specimen of indifferent blood. Combined weight of adrenals 0.395 gram.

The second adrenal specimen, obtained before injection of atropine, was stronger than 1:4,000,000 adrenalin (fig. 4, observations 4 and 8) and weaker than 1:2,700,000 (fig. 4, observation 6). Another observation (not reproduced) showed that the second specimen was somewhat weaker than 1:3,300,000. It was finally assayed at 1:3,500,000 (confirmed by a number of other observations), corresponding to an output of 0.00053 mgm. per minute for the cat, or 0.00024 mgm. per kilogram. The third specimen, collected immediately after injection of atropine was much weaker than 1:6,600,000, decidedly stronger than 1:66,000,000 and not unlike 1:20,000,000 (fig. 5, observations 47 and 49), (confirmed by other observations not reproduced). It was taken at 1:20,000,000, corresponding to an output of 0.0001 mgm. per minute for the cat, or 0.00005 mgm. per kilogram. The fourth specimen, obtained 1½ minutes after injection of atropine, was decidedly stronger than 1:13,300,000, stronger than 1:10,000,000, decidedly weaker than 1:4,000,000 and not much

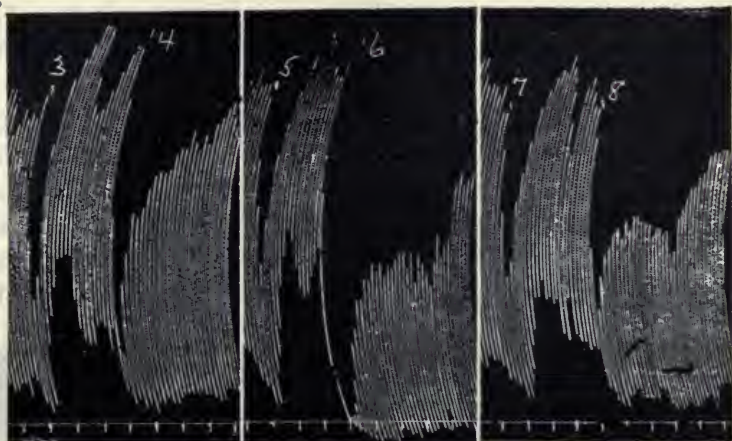


FIG. 4. INTESTINE TRACINGS. BLOODS FROM CAT 458

At 3, 5 and 7, Ringer was replaced by jugular blood and this at 4 by jugular blood to which was added adrenalin to make a concentration of 1:4,000,000; at 6 by jugular blood to which was added adrenalin to make a concentration of 1:2,700,000; at 8 by the second adrenal specimen (collected before intravenous injection of atropine). All the bloods were diluted with 3 volumes of Ringer (the adrenalin bloods after adding the adrenalin). Reduced to one-half.

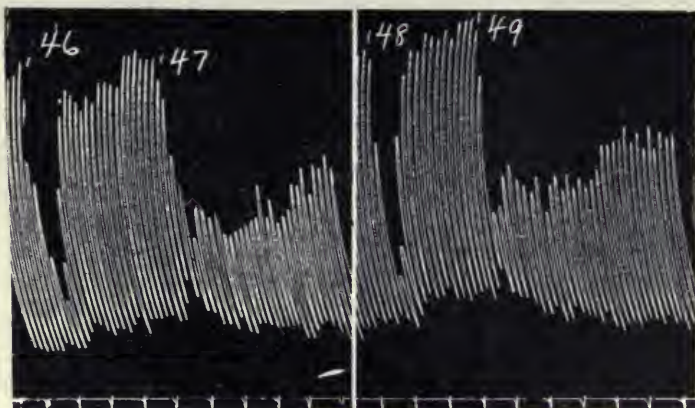


FIG. 5. INTESTINE TRACINGS. BLOODS FROM CAT 458

At 46 and 48, Ringer was replaced by indifferent blood (collected after intravenous injection of atropine) and this at 47 by the third adrenal specimen (collected immediately after injection of atropine); at 49 by indifferent blood to which was added adrenalin to make a concentration of 1:20,000,000. All the bloods were diluted with 3 volumes Ringer (the adrenalin blood after adding the adrenalin). Reduced to two-thirds.

different from 1:6,600,000 (fig. 6, observations 37 and 39). The assay at 1:6,600,000 was confirmed by other observations and corresponded to an output of 0.00023 mgm. per minute for the cat, or 0.0001 mgm. per kilogram. The sixth specimen, procured 20 minutes after injection of atropine, was much stronger than 1:4,000,000 adrenalin (fig. 7, observations 23 and 25), stronger than 1:2,700,000 (fig. 7, observation 27) and weaker than 1:1,330,000 (fig. 7, observation 29), (confirmed by three other sets of observations, not reproduced). It was assayed at

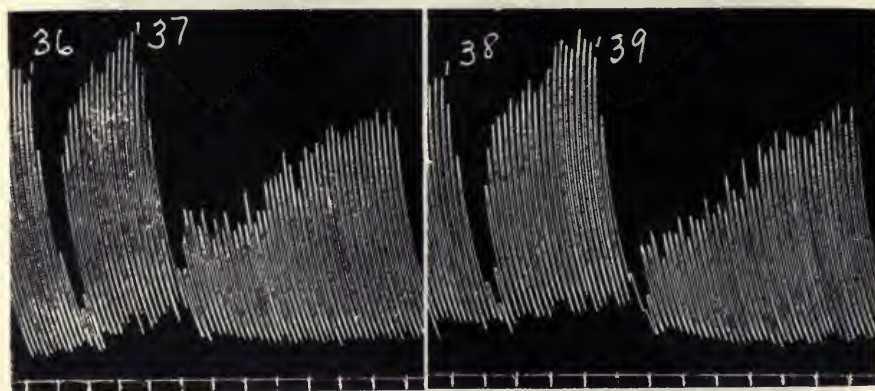


FIG. 6. INTESTINE TRACINGS. BLOODS FROM CAT 458

At 36 and 38 Ringer was replaced by indifferent blood (collected after intravenous injection of atropine) and this at 37 by the fourth adrenal specimen (collected $1\frac{1}{2}$ minutes after intravenous injection of atropine); at 39 by indifferent blood to which was added adrenalin to make a concentration of 1:6,600,000. All the bloods were diluted with 3 volumes Ringer (the adrenalin blood after adding the adrenalin). Reduced to two-thirds.

1:2,500,000, corresponding to an output of 0.0007 mgm. per minute for the cat, or 0.00032 mgm. per kilogram.

The assays of the adrenal blood specimens collected before and after administration of atropine, in this experiment, indicate a transient depression of the epinephrin output during the first few minutes following the injection of the drug. Later, however, while stimulation of the peripheral end of the vagus proved that the drug was still exerting its action on the vagus endings,

the epinephrin output of the adrenals had mounted to somewhat higher than the initial rate.

In the next experiment (cat 461) 10 mgm. of atropine sulphate was administered to a cat weighing 2.43 kgm. (4.1 mgm. per kilogram of body weight). This was the largest dose employed.

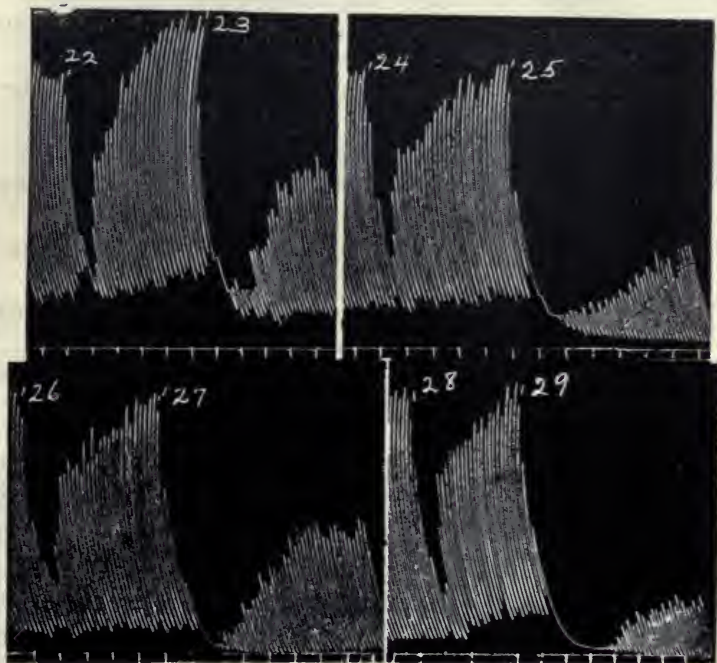


FIG. 7. INTESTINE TRACINGS. BLOODS FROM CAT 458

At 22, 24, 26 and 28, Ringer was replaced by indifferent blood (collected after intravenous injection of atropine) and this at 23 by indifferent blood to which was added adrenalin to make a concentration of 1:4,000,000; at 25 by the sixth adrenal specimen (collected 20 minutes after injection of atropine); at 27 by indifferent blood to which was added adrenalin to make a concentration of 1:2,700,000; at 29 by indifferent blood to which was added adrenalin to make a concentration of 1:1,330,000. All the bloods were diluted with 3 volumes Ringer (the adrenalin bloods after adding the adrenalin). Reduced to one-half.

Condensed protocol. Cat 461; female; weight 2.43 kgm.

Under ether inserted cannulae into the trachea, external jugular vein and carotid artery. Obtained indifferent (jugular) blood.

10.10 a.m. Cava pocket completed; collected adrenal blood.

10.12½ a.m. First specimen, 1.45 grams in 30 seconds (2.9 grams per minute).

10.13 a.m. Second specimen, 5.35 grams in 2 minutes (2.7 grams per minute); blood pressure 122 mm. of mercury.

10.25½ a.m. End of intravenous injection of 10 mgm. atropine sulphate; blood pressure 92 mm. of mercury.

10.26 a.m. Third specimen, 1.6 grams in 1 minute.

10.27 a.m. Fourth specimen, 4.5 grams in 3 minutes (1.5 grams per minute); blood pressure 60 mm. of mercury.

10.40½ a.m. Fifth specimen, 0.7 gram in 30 seconds (1.4 grams per minute).

10.41 a.m. Sixth specimen, 4 grams in 3 minutes (1.3 grams per minute); blood pressure 57 mm. of mercury.

Obtained another specimen of indifferent blood. Combined weight of adrenals 0.312 gram.

The assay of the second adrenal specimen, collected before injection of atropine, showed the concentration of epinephrin in the blood to be decidedly less than 1:14,000,000, and somewhat less than 1:28,000,000 (observations not reproduced). It was taken at 1:27,000,000, corresponding to an output of 0.0001 mgm. per minute for the cat, or 0.00004 mgm. per kilogram. This is much below the average output for cats under our experimental conditions.

The fourth specimen, obtained 1½ minutes after injection of atropine, was much weaker than 1:5,700,000 adrenalin, stronger than 1:17,000,000, and not much different from 1:11,400,000, probably slightly weaker (fig. 8, observations 47 and 49). It was assayed at 1:12,000,000 (confirmed by other observations), corresponding to an output of 0.00012 mgm. per minute for the cat, or 0.00005 mgm. per kilogram. The fourth specimen was shown to be stronger than the third in two qualitative observations. Since the blood flow was about the same in both specimens the output during collection of the third specimen must have been somewhat less than during collection of the fourth.

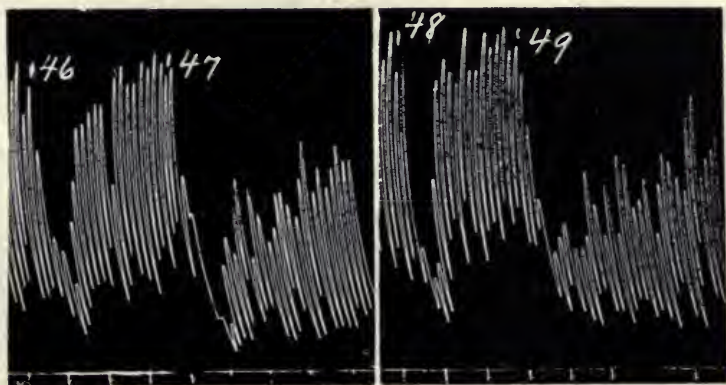


FIG. 8. INTESTINE TRACINGS. BLOODS FROM CAT 461

At 46 and 48 Ringer was replaced by indifferent blood (collected after intravenous injection of atropine) and this at 47 by indifferent blood to which was added adrenalin to make a concentration of 1:11,400,000; at 49 by the fourth adrenal specimen (collected 1½ minutes after injection of atropine). All the bloods were diluted with 3 volumes Ringer (the adrenalin blood after adding the adrenalin). Reduced to three-fourths.

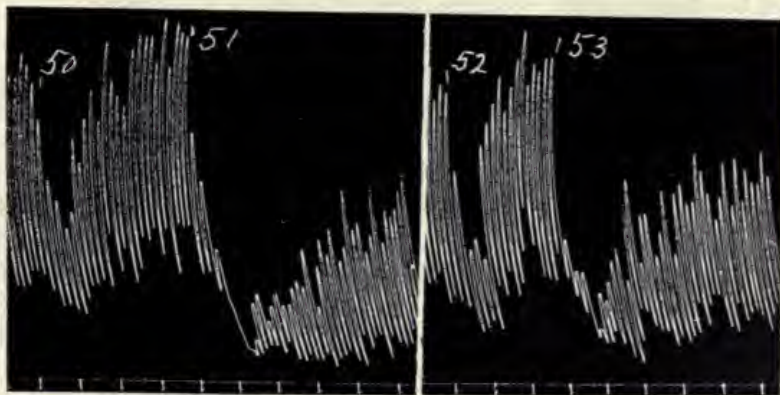


FIG. 9. INTESTINE TRACINGS. BLOODS FROM CAT 461

At 50 and 52, Ringer was replaced by indifferent blood (collected after intravenous injection of atropine) and this at 51 by indifferent blood to which was added adrenalin to make a concentration of 1:5,700,000; at 53 by the sixth adrenal specimen (collected 15 minutes after injection of atropine). All the bloods were diluted with 3 volumes Ringer (the adrenalin blood after adding the adrenalin). Reduced to three-fourths.

The sixth specimen, procured 15 minutes after injection of atropine, was much stronger than 1:11,400,000 adrenalin, decidedly weaker than 1:4,300,000 and somewhat less than 1:5,700,000 (fig. 9, observations 51 and 53). After confirmation by a number of other observations it was assayed at 1:6,000,000, corresponding to an output of 0.00022 mgm. per minute for the cat, or 0.0001 mgm. per kilogram.

The initial output of adrenal epinephrin, before administration of atropine was unusually low (about one-fourth of the normal average in etherized cats, under our experimental conditions). It is possible that if any depression of the output was caused by the drug it could not manifest itself because of the already very low rate of epinephrin secretion existing. In the sixth specimen, procured 15 minutes after injection of atropine, the epinephrin concentration indicated an output twice as high as the initial rate.

In the next experiment (cat 460) the dose of atropine injected was 1.6 mgm. per kilogram of body weight. The cat had given birth to a litter of kittens six weeks previously. Urethane was used for anesthesia in this experiment, ether in the others.

Condensed protocol. Cat 460; female; weight 3.08 kgm.

Under urethane inserted cannulae into the trachea, external jugular vein, and carotid artery. Obtained indifferent (jugular) blood.

11.00 a.m. Cava pocket completed. Collected adrenal blood.

11.01½ a.m. First specimen, 2.15 grams in 30 seconds (4.3 grams per minute).

11.02 a.m. Second specimen, 5.4 grams in 90 seconds (3.6 grams per minute); blood pressure 116 mm. of mercury.

11.05½ a.m. End of intravenous injection of 5 mgm. atropine sulphate; blood pressure 92 mm. of mercury.

11.06 a.m. Third specimen, 2.65 grams in 1 minute.

11.07 a.m. Fourth specimen, 4.65 grams in 2 minutes (2.3 grams per minute); blood pressure 83 mm. of mercury.

11.20 a.m. Stimulation of peripheral end of vagus caused no change in heart rate or blood pressure (72 mm. of mercury).

11.24 a.m. Fifth specimen, 1.15 grams in 30 seconds (2.3 grams per minute).

11.24½ a.m. Sixth specimen, 4.75 grams in 2 minutes (2.37 grams per minute); blood pressure 72 mm. of mercury.

Another specimen of indifferent blood was obtained. Combined weight of adrenals 0.484 gram.

The second adrenal specimen, collected before injection of atropine, was decidedly stronger than 1:4,300,000 adrenalin, stronger than 1:3,550,000, weaker than 1:2,100,000, and not very different from 1:2,850,000. A number of observations confirmed the final assay at 1:2,800,000, corresponding to an output of 0.0013 mgm. per minute for the cat, or 0.0004 mgm. per kilogram. The third specimen, obtained immediately after injection of atropine, was assayed at 1:6,000,000, corresponding to an output of 0.00044 mgm. per minute for the cat, or 0.00014 mgm. per kilogram. The fourth specimen, collected 1½ minutes after injection of atropine, was stronger than 1:5,700,000, and decidedly weaker than 1:2,850,000. The final assay showed it to be equal to 1:4,000,000, corresponding to an output of 0.0006 mgm. per minute for the cat, or 0.0002 mgm. per kilogram. The sixth specimen, obtained 19 minutes after injection of atropine, was decidedly stronger than 1:5,700,000 adrenalin, somewhat stronger than 1:2,850,000, and not quite as strong as 1:2,100,000. It was finally assayed at 1:2,400,000, corresponding to an output of 0.001 mgm. per minute for the cat, or 0.00032 mgm. per kilogram.

In this animal the initial output of epinephrin from the adrenals, before administration of atropine was higher than the average rate found by us in cats under urethane. There was a moderate transient depression of the epinephrin output, after the injection of atropine, returning to practically the initial rate within 20 minutes. As the rate of output was already high before atropine was injected, it is likely that a moderate increase in the rate that might have been caused by the drug could not be manifested.

Summing up the results of our experiments it will be seen that atropine does not produce a very marked effect on the rate of liberation of epinephrin from the adrenal glands. The drug does not cause complete suppression nor a marked prolonged depres-

sion of the epinephrin output. In fact, the experiments indicate that there may be a moderate increase which, when large doses are employed, may be preceded by a moderate transient depression of the rate of output, under the influence of atropine.

THE INFLUENCE OF PILOCARPINE ON THE EPINEPHRIN OUTPUT

One experiment was made with subcutaneous injection, the others with intravenous administration of pilocarpine. The dose employed was approximately 1 mgm. per kilogram of body weight in all except one cat, which received 0.1 mgm. per kilogram. A number of protocols and some of the tracings used in the assay follow.

Condensed protocol. Cat 287; male; weight 3.715 kgm.

Under ether inserted cannulae into trachea, carotid artery and external jugular vein; obtained indifferent (jugular) blood.

12.22 p.m. Cava pocket completed; collected adrenal blood.

12.24½ p.m. First specimen, 1.4 grams in 45 seconds (1.9 grams per minute).

12.25 p.m. Second specimen, 4.8 grams in 3 minutes (1.6 grams per minute); blood pressure 92 mm. of mercury.

12.33 p.m. End of intravenous injection of 3 mgm. pilocarpine hydrochloride; blood pressure 72 mm. of mercury. A profuse salivary and lachrymal secretion was evoked, which continued throughout the experiment, and the pupils dilated widely.

12.34 p.m. Third specimen, 2.75 grams in 2 minutes (1.4 grams per minute).

12.36 p.m. Fourth specimen, 3.6 grams in 4 minutes (0.9 gram per minute); blood pressure fell from 76 mm. at beginning of collection to 60 mm. of mercury at end.

Obtained another specimen of jugular blood.

12.50 p.m. Fifth specimen, 0.6 gram in 1 minute.

12.51 p.m. Sixth specimen, 2.6 grams in 5 minutes (0.5 gram per minute); blood pressure 49 mm. of mercury.

Another specimen of indifferent blood was now obtained. Combined weight of adrenals 0.376 gram.

The proportion of serum in the blood was determined by the electrical conductivity method at 44.7 per cent. By the haematocrite, after 5 minutes rotation 30 per cent, after 15 minutes 40 per cent, after 22 minutes 41 per cent and after 30 minutes 42 per cent.

The second adrenal specimen, collected before injection of pilocarpine, was weaker than 1:5,000,000 adrenalin (fig. 10, observations 11 and 13) and somewhat stronger than 1:7,500,000

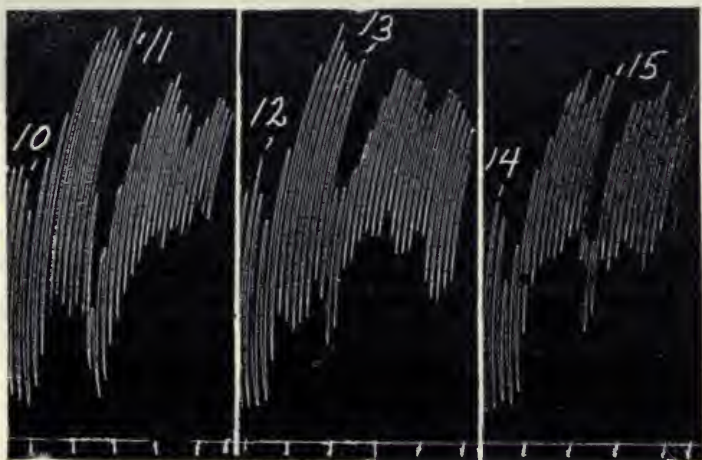


FIG. 10. INTESTINE TRACINGS. BLOODS FROM CAT 287

At 10, 12, and 14, Ringer was replaced by jugular blood and this at 11 by jugular blood to which was added adrenalin to make a concentration of 1:5,000,000; at 13 by the second adrenal specimen (collected before intravenous injection of pilocarpine); at 15 by jugular blood to which was added adrenalin to make a concentration of 1:7,500,000. All the bloods were diluted with 3 volumes of Ringer (the adrenalin bloods after adding the adrenalin). Reduced to three-fourths.

(fig. 10, observation 15). Another set of observations verified the assay at 1:6,500,000, corresponding to an output of 0.00035 mgm. per minute for the cat, or 0.0001 mgm. per kilogram.

The fourth specimen, obtained 3 minutes after injection of pilocarpine, was decidedly stronger than 1:2,500,000, somewhat stronger than 1:1,300,000 (fig. 11, observations 33, 37 and 39) and decidedly weaker than 1:930,000. It was finally assayed

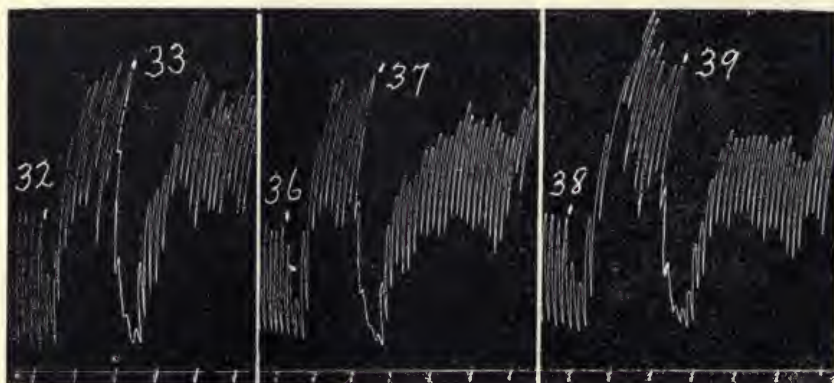


FIG. 11. INTESTINE TRACINGS. BLOODS FROM CAT 287

At 32, 36 and 38, Ringer was replaced by indifferent blood (collected after intravenous injection of pilocarpine), and this at 33 by indifferent blood to which was added adrenalin to make a concentration of 1:2,500,000; at 37 by indifferent blood to which was added adrenalin to make a concentration of 1:1,300,000; at 39 by the fourth adrenal specimen (collected 3 minutes after intravenous injection of pilocarpine). All the bloods were diluted with 3 volumes Ringer (the adrenalin bloods after adding the adrenalin). Reduced to two-thirds.

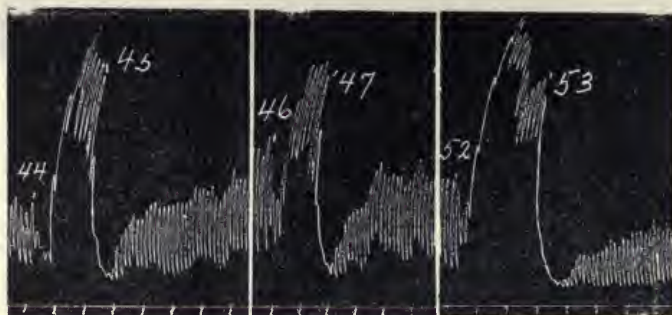


FIG. 12. INTESTINE TRACINGS. BLOODS FROM CAT 287

At 44, 46 and 52, Ringer was replaced by indifferent blood (collected after intravenous injection of pilocarpine); and this at 45 by the sixth adrenal specimen (collected 18 minutes after intravenous injection of pilocarpine); at 47 by indifferent blood to which was added adrenalin to make a concentration of 1:650,000; at 53 by indifferent blood to which was added adrenalin to make a concentration of 1:375,000. All the bloods were diluted with 3 volumes Ringer (the adrenalin bloods after adding the adrenalin). Reduced to one-half.

at 1:1,200,000 (corroborated by two other sets of observations), corresponding to an output of 0.00075 mgm. per minute for the cat, or 0.0002 mgm. per kilogram.

The sixth specimen, procured 18 minutes after injection of pilocarpine, was shown to be decidedly stronger than 1:875,000 adrenalin, stronger than 1:650,000 (fig. 12, observations 45 and 47), weaker than 1:375,000 (fig. 12, observation 53), and not much different from 1:500,000 (verified by two sets of observations not reproduced). The final assay at 1:500,000 corresponded to an output of 0.001 mgm. per minute for the cat, or 0.00027 mgm. per kilogram. This concentration would correspond to 1:225,000 for the serum, which is somewhat greater than is usually found in the absence of influence of drugs.

The initial rate, before pilocarpine was administered, was somewhat lower than the average output in cats under ether and the drug apparently doubled the output in this animal, although the rate per minute remained within the normal range under our experimental conditions.

Condensed protocol. Cat 289; female, weight 2.83 kgm.

Under ether inserted cannulae into trachea, carotid artery and external jugular vein; obtained indifferent (jugular) blood.

11.45 a.m. Cava pocket completed; collected adrenal blood.

11.47 a.m. First specimen, 1.2 grams in 30 seconds (2.4 grams per minute).

11.47½ a.m. Second specimen, 6 grams in 3 minutes (2 gram per minute); blood pressure 97 mm. of mercury.

11.55 a.m. Started artificial respiration.

11.58¼ a.m. End of intravenous injection of 3 mgm. pilocarpine hydrochloride; blood pressure 50 mm. of mercury. Profuse salivary and lachrymal secretion was evoked and pupils dilated widely, which continued throughout the experiment.

11.59 a.m. Third specimen, 2.3 grams in 3 minutes (0.8 gram per minute).

12.02 p.m. Fourth specimen, 3.65 grams in 7 minutes (0.52 gram per minute); blood pressure 44 mm. of mercury.

Another specimen of indifferent blood was now obtained. Combined weight of adrenals 0.342 gram. The proportion of serum in the blood was 72.3 per cent, determined by the electrical conductivity method.

The assay on intestine segments showed that the second adrenal specimen, collected before injection of pilocarpine was much stronger than 1:4,000,000 adrenalin, decidedly stronger than 1:2,700,000, stronger than 1:2,000,000 (fig. 13, observations 21 and 25), and weaker than 1:1,000,000 (fig. 13, observation 23). The final assay (confirmed by a number of other observations) at

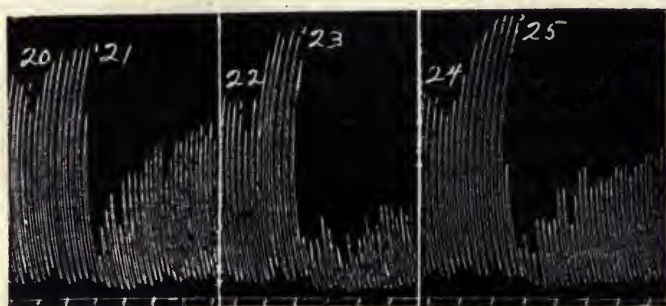


FIG. 13. INTESTINE TRACINGS. BLOODS FROM CAT 289

At 20, 22 and 24, Ringer was replaced by jugular blood and this at 21 by jugular blood to which was added adrenalin to make a concentration of 1:2,000,000 at 23 by jugular blood to which was added adrenalin to make a concentration of 1:1,000,000; at 25 by the second adrenal specimen (collected before intravenous injection of pilocarpine). All the bloods were diluted with 3 volumes of Ringer (the adrenalin bloods after adding the adrenalin). Reduced to one-half.

1:1,300,000 corresponded to an output of 0.0015 mgm. per minute for the cat, or 0.0005 mgm. per kilogram. This is greater than the normal average usually found in etherized cats, under our experimental conditions.

The third specimen, obtained immediately after injection of pilocarpine, was decidedly weaker than 1:270,000 (fig. 14, observations 51 and 55), and somewhat stronger than 1:660,000 (fig. 14, observation 53). With the aid of numerous other observations it was assayed at 1:600,000, corresponding to an out-

put of 0.0013 mgm. per minute for the cat, or 0.00046 mgm. per kilogram. The fourth specimen, collected 3 minutes after injection of pilocarpine, was decidedly stronger than 1:660,000 (fig. 14, observations 47 and 53), stronger than 1:400,000 (fig. 14, observation 49) and weaker than 1:270,000 (fig. 14, observation 55). Two other sets of observations (not reproduced) verified

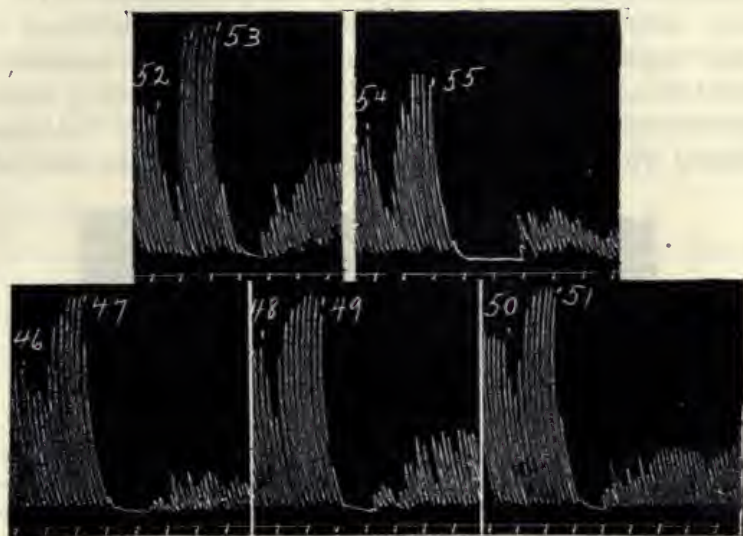


FIG. 14. INTESTINE TRACINGS. BLOODS FROM CAT 289

At 46, 48, 50, 52 and 54, Ringer was replaced by indifferent blood (collected after intravenous injection of pilocarpine), and this at 47 by the fourth adrenal specimen (collected 3 minutes after injection of pilocarpine); at 49 by indifferent blood to which was added adrenalin to make a concentration of 1:400,000; at 51 by the third adrenal specimen (collected immediately after injection of pilocarpine); at 53 by indifferent blood to which was added adrenalin to make a concentration of 1:660,000; at 55 by indifferent blood to which was added adrenalin to make a concentration of 1:270,000. All the bloods were diluted with 5 volumes Ringer (the adrenalin bloods after adding the adrenalin). Reduced to one-half.

the assay at 1:350,000, corresponding to an output of 0.0015 mgm. per minute for the cat or 0.0005 mgm. per kilogram. The epinephrin concentration in the fourth specimen was apparently increased beyond the usual maximum under the influence of

pilocarpine and had the blood flows, during collection of the third and fourth specimens, been larger it is possible that a calculated increase in output might have been gotten. It is probable, however, that the drug could not exhibit an increase over the already high initial output existing before injection of pilocarpine.

Qualitative (and frequently quantitative) confirmation of the assays made on segments of intestine are usually obtained with uterus segments. As it is obviously impossible to reproduce all of the tracings used in assays involving a large number of repeated observations, only one figure is given as a sample of the tracings obtained with the uterus. Figure 15 shows that the third adre-

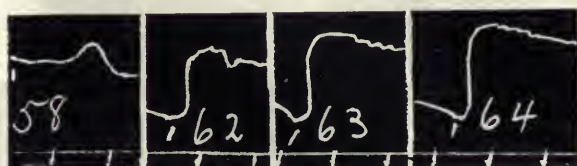


FIG. 15. UTERUS TRACINGS. BLOODS FROM CAT 289

At 58, Ringer was replaced by indifferent blood (collected after intravenous injection of pilocarpine); at 62 by the second adrenal specimen (collected before injection of pilocarpine); at 63 by the third adrenal specimen (collected immediately after injection of pilocarpine); at 64 by the fourth adrenal specimen (collected 3 minutes after injection of pilocarpine). All the bloods were diluted with 6 volumes of Ringer.

nal specimen (observation 63) caused a greater increase in tone than the second (observation 62), and the fourth (observation 64), greater than either of the others. All of the adrenal specimens caused a much larger increase in tone than the corresponding indifferent blood (observation 58). When the epinephrin concentration, in the blood tested, is high, a maximal reaction is ordinarily obtained. For proper qualitative comparisons, therefore, it is usually necessary to dilute the specimen, before applying to the segment, until the reaction obtained is sub-maximal.

Condensed protocol. Cat 463; female; weight 2.87 kgm.

Under ether inserted cannulae into trachea, external jugular vein and carotid artery. Obtained indifferent (jugular) blood.

9.40 a.m. Cava pocket completed; collected adrenal blood.

9.41½ a.m. First specimen, 2.25 grams in 30 seconds (5.5 grams per minute).

9.42 a.m. Second specimen, 5.7 grams in 90 seconds (3.8 grams per minute); blood pressure 84 mm. of mērcury.

9.50½ a.m. End of intravenous injection of 3 mgm. pilocarpine hydrochloride; blood pressure 76 mm. of mercury. Profuse salivary and lachrymal secretion, and pupils dilated widely, which continued throughout the experiment.

9.51 a.m. Third specimen, 2.95 grams in 1 minute.

9.52 a.m. Fourth specimen, 7 grams in 2 minutes (3.5 grams per minute); blood pressure 92 mm. of mercury.

10.07½ a.m. Fifth specimen, 1.25 grams in 30 seconds (2.5 grams per minute).

10.08 a.m. Sixth specimen, 6.1 grams in 3 minutes (2 grams per minute); blood pressure 64 mm. of mercury.

Another specimen of indifferent blood was obtained. Combined weight of adrenals 0.341 gram.

The second specimen, collected before injection of pilocarpine, was decidedly stronger than 1:13,000,000 adrenalin, much weaker than 1:4,000,000, weaker than 1:5,300,000 and somewhat weaker than 1:6,660,000. Repeated observations confirmed the assay at 1:7,000,000, corresponding to an output of 0.00054 mgm. per minute for the cat, or 0.00019 mgm. per kilogram. The third specimen, collected immediately after injection of pilocarpine, was stronger than 1:6,660,000, and decidedly weaker than 1:2,700,000. It was finally assayed at 1:5,300,000, corresponding to an output of 0.0006 mgm. per minute for the cat, or 0.0002 mgm. per kilogram. The fourth specimen, obtained 1½ minutes after injection of pilocarpine, was stronger than 1:5,300,000, decidedly weaker than 1:2,700,000 and somewhat weaker than 1:4,000,000. The final assay proved it to be equal to 1:4,500,000, corresponding to an output of

0.0008 mgm. per minute for the cat, or 0.00028 mgm. per kilogram.

The sixth specimen, obtained 18 minutes after injection of pilocarpine, was decidedly stronger than 1:5,300,000, stronger than 1:4,000,000, weaker than 1:2,000,000, and not unlike 1:2,700,000. The assay at 1:2,700,000 was verified with a number of observations and corresponded to an output of 0.00075 mgm. per minute for the cat, or 0.00025 mgm. per kilogram.

In the next experiment (cat 464) the animal was anesthetized with urethane.

Condensed protocol. Cat 464: male; weight 2.85 kgm.

Under urethane inserted cannulae into trachea, external jugular vein and carotid artery. Obtained indifferent (jugular) blood.

10.20 a.m. Cava pocket completed; collected adrenal blood.

10.20½ a.m. First specimen, 1.4 grams in 30 seconds (2.8 grams per minute).

10.22 a.m. Second specimen, 4.55 grams in 2 minutes (2.3 grams per minute); blood pressure 97 mm. of mercury.

10.30½ a.m. End of intravenous injection of 4 mgm. pilocarpine hydrochloride; blood pressure 72 mm. of mercury. Profuse salivary and lachrymal secretion evoked and pupils dilated widely, which continued throughout the experiment.

10.31 a.m. Third specimen, 3.5 grams in 1 minute.

10.32 a.m. Fourth specimen, 5.6 grams in 2 minutes (2.8 grams per minute); blood pressure 110 mm. of mercury.

10.45 a.m. Stimulation of peripheral end of vagus caused no change in heart rate or blood pressure (98 mm. of mercury).

10.50 a.m. Fifth specimen, 1.25 grams in 30 seconds (2.5 grams per minute).

10.50½ a.m. Sixth specimen, 4.75 grams in 2½ minutes (1.9 grams per minute); blood pressure 88 mm. of mercury.

Another specimen of indifferent blood was obtained. Combined weight of adrenals 0.518 gram.

The second adrenal specimen, obtained before injection of pilocarpine, was decidedly stronger than 1:6,660,000 adrenalin, weaker than 1:4,700,000, decidedly weaker than 1:4,000,000.

Numerous observations showed it to be equal to 1:5,500,000, corresponding to an output of 0.0004 mgm. per minute for the cat, or 0.00015 mgm. per kilogram. The third specimen, collected immediately after injection of pilocarpine, was assayed at 1:20,000,000, corresponding to an output of 0.00017 mgm. per minute for the cat, or 0.00005 mgm. per kilogram. The fourth specimen, obtained 1½ minutes after injection of pilocarpine, was decidedly stronger than 1:6,660,000, weaker than 1:4,000,000, somewhat weaker than 1:5,300,000 and not different from 1:5,000,000. It was finally assayed at 1:5,000,000, corresponding to an output of 0.00056 mgm. per minute for the cat, or 0.0002 mgm. per kilogram. The sixth specimen, obtained 20 minutes after injection of pilocarpine was decidedly weaker than 1:1,300,000, decidedly stronger than 1:5,300,000, much stronger than 1:4,000,000, and not far from 1:2,700,000. It was finally assayed at 1:2,900,000, corresponding to an output of 0.00065 mgm. per minute for the cat, or 0.00022 mgm. per kilogram.

Condensed protocol. Cat 467; female; weight 2.43 kgm.

Under ether inserted cannulae into trachea, external jugular vein, and carotid artery. Obtained indifferent (jugular) blood.

9.55 a.m. Cava pocket completed; collected adrenal blood.

10.00½ a.m. First specimen 0.85 grams in 30 seconds (1.7 grams per minute).

10.01 a.m. Second specimen, 3.9 grams in 3 minutes (1.3 grams per minute); blood pressure 100 mm. of mercury.

10.07¾ a.m. End of intravenous injection of 2.5 mgm. pilocarpine hydrochloride. The pupils dilated widely, and profuse salivary and lachrymal secretion was evoked, lasting throughout the experiment; blood pressure 123 mm. of mercury.

10.08 a.m. Third specimen, 1.95 grams in 1 minute.

10.09 a.m. Fourth specimen, 4.15 grams in 2½ minutes (1.66 grams per minute); blood pressure 118 mm. of mercury.

10.25 a.m. Fifth specimen, 0.45 gram in 30 seconds (0.9 gram per minute).

10.25½ a.m. Sixth specimen, 2.45 grams in 4 minutes (0. ram per minute); blood pressure 56 mm. of mercury.

Another specimen of indifferent blood was obtained. Combined weight of adrenals 0.502 gram.

The assay showed that the second adrenal specimen, collected before injection of pilocarpine, was weaker than 1:3,750,000 adrenalin, much stronger than 1:7,500,000, stronger than 1:6,250,000, not far from 1:5,000,000. It was finally assayed at 1:5,500,000, corresponding to an output of 0.00024 mgm. per minute for the cat, or 0.0001 mgm. per kilogram. The third specimen, collected just after injection of pilocarpine, was much stronger than 1:18,750,000, decidedly weaker than 1:10,000,000, and somewhat weaker than 1:12,500,000. It was finally assayed at 1:14,000,000, corresponding to an output of 0.00014 mgm. per minute for the cat, or 0.00006 mgm. per kilogram. The fourth specimen, collected 1½ minutes after injection of pilocarpine, was not quite as strong as 1:7,500,000 and stronger than 1:10,000,000. It was assayed at 1:8,000,000, corresponding to an output of 0.00021 mgm. per minute for the cat, or 0.00009 mgm. per kilogram. The sixth specimen, procured 18 minutes after injection of pilocarpine, was much stronger than 1:3,750,000 adrenalin, stronger than 1:2,500,000, and not very different from 1:2,000,000. It was assayed at 1:2,000,000, corresponding to an output of 0.0003 mgm. per minute for the cat, or 0.00014 mgm. per kilogram.

In the next experiment (cat 465), a much smaller dose of pilocarpine was administered (0.1 mgm. per kilogram of body weight).

Condensed protocol. Cat 465; female; weight 2.86 kgm.

Under ether inserted cannulae into trachea, external jugular vein and carotid artery. Obtained indifferent (jugular) blood.

9.45 a.m. Cava pocket completed; collected adrenal blood.

9.46½ a.m. First specimen, 1.2 grams in 30 seconds (2.4 grams per minute).

9.47 a.m. Second specimen, 4.25 grams in 2 minutes (2.1 grams per minute); blood pressure 124 mm. of mercury.

- 10.10 $\frac{1}{2}$ a.m. End of intravenous injection of 0.3 mgm. pilocarpine hydrochloride; blood pressure 94 mm. of mercury. The pupils dilated widely and profuse salivary and lachrymal secretion was evoked, lasting throughout the experiment.
- 10.11 a.m. Third specimen, 3.8 grams in 1 minute.
- 10.12 a.m. Fourth specimen, 6.85 grams in 2 minutes (3.4 grams per minute); blood pressure 104 mm. of mercury.
- 10.20 a.m. Stimulation of peripheral end of vagus caused no change in heart rate or blood pressure (94 mm. of mercury).
- 10.25 a.m. Fifth specimen, 1.3 grams in 30 seconds (2.6 grams per minute).
- 10.25 $\frac{1}{2}$ a.m. Sixth specimen, 6.2 grams in 3 minutes (2.1 grams per minute); blood pressure 78 mm. of mercury.

Another specimen of indifferent blood was obtained. Combined weight of adrenals 0.405 gram.

The epinephrin concentration in the second specimen, obtained before injection of pilocarpine, was decidedly greater than 1:6,250,000, greater than 1:5,000,000 (fig. 16, observations 14 and 16), much less than 1:3,125,000, and not as great as 1:3,750,000 (fig. 16, observation 12). It was finally assayed at 1:4,300,000 (corroborated by four sets of observations), corresponding to an output of 0.0005 mgm. per minute for the cat, or 0.00018 mgm. per kilogram. The concentration of epinephrin in the third specimen, collected immediately after injection of pilocarpine, was approximately the same as that in the fourth specimen, collected 1 $\frac{1}{2}$ minutes after injection of pilocarpine (fig. 17, observations 26 and 28). Both specimens were somewhat stronger than 1:7,500,000 (fig. 17, observation 32), and weaker than 1:6,250,000 (fig. 17, observation 34). A number of other observations verified the assay at 1:7,000,000, corresponding to an output of 0.00054 mgm. per minute for the cat, or 0.00019 mgm. per kilogram for the third specimen and 0.00048 mgm. per minute for the cat, or 0.00017 mgm. per kilogram for the fourth specimen. The sixth specimen, collected 15 minutes after injection of pilocarpine, was decidedly stronger than 1:9,375,000, stronger than 1:7,500,000, weaker than 1:5,000,000, and somewhat weaker than

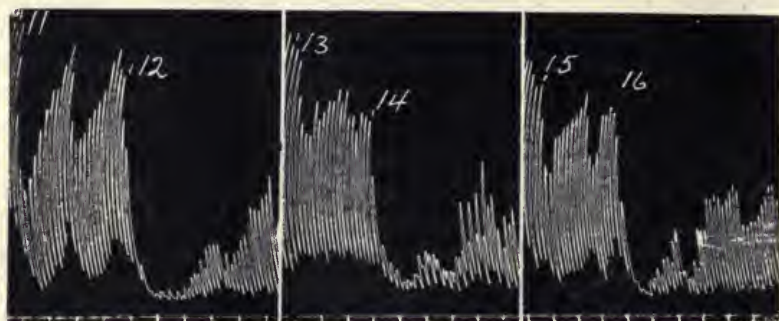


FIG. 16. INTESTINE TRACINGS. BLOODS FROM CAT 465

At 11, 13 and 15, Ringer was replaced by jugular blood and this at 12 by jugular blood to which was added adrenalin to make a concentration of 1:3,750,000; at 14 by the second adrenal specimen (collected before intravenous injection of pilocarpine); at 16 by jugular blood to which was added adrenalin to make a concentration of 1:5,000,000. All the bloods were diluted with 3 volumes Ringer (the adrenalin bloods after adding the adrenalin). Reduced to one-half.

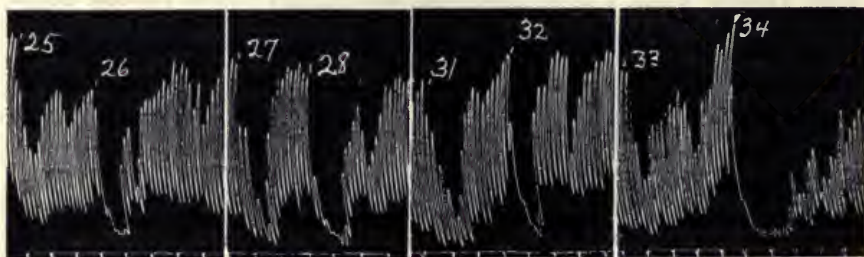


FIG. 17. INTESTINE TRACINGS. BLOODS FROM CAT 465

At 25, 27, 31 and 33, Ringer was replaced by indifferent blood (collected after intravenous injection of pilocarpine), and this at 26 by the fourth adrenal specimen (collected 1½ minutes after injection of pilocarpine); at 28 by the third adrenal specimen (collected immediately after injection of pilocarpine); at 32 by indifferent blood to which was added adrenalin to make a concentration of 1:7,500,000; at 34 by indifferent blood to which was added adrenalin to make a concentration of 1:6,250,000. All the bloods were diluted with 3 volumes Ringer (the adrenalin bloods after adding the adrenalin). Reduced to one-half.

1:6,250,000 (fig. 18 observations 36 and 38). It was finally assayed at 1:6,500,000 (corroborated by three other sets of observations), corresponding to an output of 0.00032 mgm. per minute for the cat, or 0.00011 mgm. per kilogram.

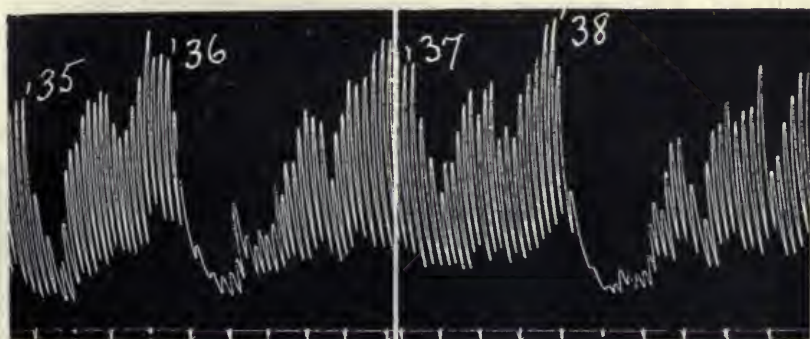


FIG. 18. INTESTINE TRACINGS. BLOOD FROM CAT 465

At 35 and 37, Ringer was replaced by indifferent blood (collected after intravenous injection of pilocarpine), and this at 36 by the sixth adrenal specimen (collected 15 minutes after injection of pilocarpine); at 38 by indifferent blood to which was added adrenalin to make a concentration of 1:6,250,000. All the bloods were diluted with 3 volumes Ringer (the adrenalin blood after adding the adrenalin). Reduced to three-fourths.

In the following experiment, pilocarpine was administered subcutaneously.

Condensed protocol. Cat 466; female; weight 2.77 kgm.

Under ether inserted cannulae into trachea, external jugular vein and carotid artery. Obtained indifferent (jugular) blood.

9.40 a.m. Cava pocket completed; collected adrenal blood.

9.45½ a.m. First specimen, 2.2 grams in 30 seconds (4.4 grams per minute).

9.46 a.m. Second specimen, 5.65 grams in 90 seconds (3.8 grams per minute); blood pressure 114 mm. of mercury.

9.53 to 9.54 a.m. Subcutaneous injection of 3 mgm. pilocarpine hydrochloride. The pupils dilated widely and profuse salivary and lachrymal secretion was evoked lasting throughout the experiment; blood pressure at end of injection 90 mm. of mercury.

- 9.54 a.m. Third specimen, 3.5 grams in 1 minute.
9.55 a.m. Fourth specimen, 5.2 grams in 2 minutes (2.6 grams per minute); blood pressure 75 mm. of mercury.
10.02 a.m. Stimulation of peripheral end of vagus caused no change in heart rate or blood pressure (67 mm. of mercury).
10.10 a.m. Fifth specimen, 0.7 gram in 30 seconds (1.4 grams per minute).
10.10½ a.m. Sixth specimen, 3.1 grams in 3 minutes (1 gram per minute); blood pressure 47 mm. of mercury.

Another specimen of indifferent blood was obtained. Combined weight of adrenals 0.277 gram.

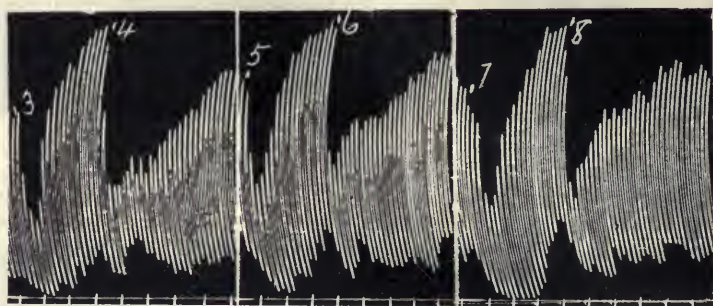


FIG. 19. INTESTINE TRACINGS. BLOODS FROM CAT 466

At 3, 5 and 7, Ringer was replaced by jugular blood and this at 4 by jugular blood to which was added adrenalin to make a concentration of 1:6,660,000; at 6 by jugular blood to which was added adrenalin to make a concentration of 1:13,300,000; at 8 by the second adrenal specimen (collected before subcutaneous injection of pilocarpine). All the bloods were diluted with 3 volumes Ringer (the adrenalin bloods after adding the adrenalin). Reduced to one-half.

The second adrenal specimen, obtained before injection of pilocarpine, was decidedly less than 1:5,300,000, less than 1:6,660,000 (fig. 19, observations 4 and 8), and greater than 1:13,300,000 (fig. 19, observation 6). The final assay was confirmed by a number of other observations at 1:9,500,000, corresponding to an output of 0.0004 mgm. per minute for the cat, or 0.00015 mgm. per kilogram.

The fourth specimen, collected 1 minute after injection of pilocarpine, was stronger than 1:5,300,000 adrenalin (fig. 20,

observations 52 and 54), and not quite as strong as 1:4,000,000. It was assayed at 1:4,600,000 (corroborated by two sets of observations), corresponding to an output of 0.00055 mgm. per minute for the cat, or 0.0002 mgm. per kilogram.

The sixth specimen, procured 16 minutes after injection of pilocarpine, was decidedly stronger than 1:2,700,000, somewhat stronger than 1:2,250,000 adrenalin (fig. 21, observations 46 and 48) and weaker than 1:1,330,000 (fig. 21, observation 50).

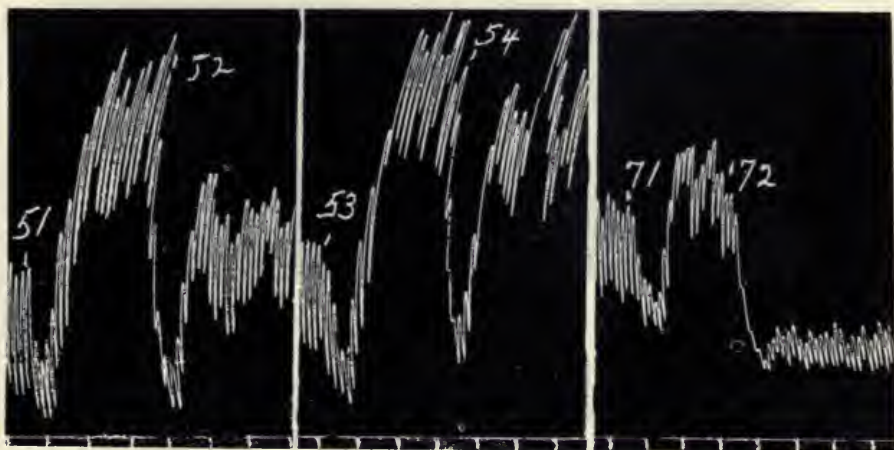


FIG. 20. INTESTINE TRACINGS. BLOODS FROM CAT 466

At 51, 53 and 71, Ringer was replaced by indifferent blood (collected after subcutaneous injection of pilocarpine) and this at 52 and at 72 by the fourth adrenal specimen (collected 1 minute after injection of pilocarpine); at 54 by indifferent blood to which was added adrenalin to make a concentration of 1:5,300,000. Before observation 71-72 the segment had been subjected to the action of atropine. All the bloods were diluted with 3 volumes Ringer (the adrenalin bloods after adding the adrenalin). Reduced to three-fourths.

Three other sets of observations corroborated the assay at 1:2,000,000, corresponding to an output of 0.0005 mgm. per minute for the cat or 0.00018 mgm. per kilogram.

A very interesting point in technique is illustrated in figure 20, observations 52 and 72. Both tracings were obtained with the same segment of intestine, subjected to the action of the same adrenal blood (fourth specimen), at 52 before atropinizing and

at 72 after atropine had been applied to the segment. We have repeatedly observed that subjecting the segment of intestine to the action of atropine renders it much more sensitive to the action of epinephrin and that specimens which have an epinephrin concentration too low to cause a definite inhibition of tone (or suppression of beats) before atropine is applied, cause a good inhibition after atropinizing the segment, or where a good reaction was obtained before atropine was used a much greater inhi-

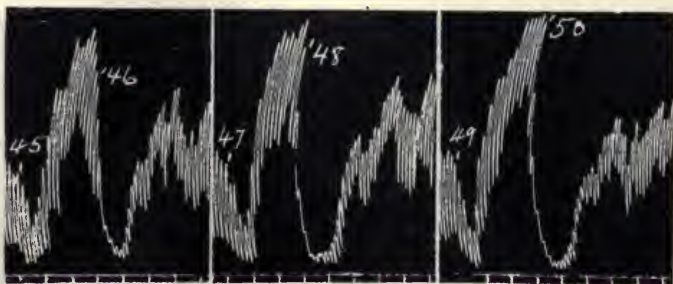


FIG. 21. INTESTINE TRACINGS. BLOODS FROM CAT 466

At 45, 47, and 49, Ringer was replaced by indifferent blood (collected after subcutaneous injection of pilocarpine); and this at 46 by indifferent blood to which was added adrenalin to make a concentration of 1:2,250,000; at 48 by the sixth adrenal specimen (collected 16 minutes after subcutaneous injection of pilocarpine); at 50 by indifferent blood to which was added adrenalin to make a concentration of 1:1,330,000. All the bloods were diluted with 3 volumes Ringer (the adrenalin bloods after adding the adrenalin). Reduced to one-half.

bition (and suppression of beats) is caused by the same specimen after the application of atropine as illustrated in figure 20. Generally, although not always, the first application of atropine causes a temporary inhibition of the contractions. The beats usually become smaller, but frequently increase after a few applications of blood to the same magnitude as before the atropine.

In nearly all of the experiments with pilocarpine, the adrenal blood specimens were first assayed on the non-atropinized segment, then again on the same segment after subjecting it to the influence of atropine.

THE INFLUENCE OF PILOCARPINE ON THE EPINEPHRINE STORE OF THE ADRENALS

One experiment was made (cat 302) on the influence of pilocarpine on the store of epinephrin in the adrenals. The left adrenal had been denervated and the left superior cervical ganglion excised two weeks previous to the experiment. Thirty-one milligrams of pilocarpine hydrochloride was injected (in doses of 5 to 10 mgm.) within a period of 5 hours. The glands were assayed colorimetrically by the method of Folin, Cannon and Denis (12). The condensed protocol of the experiment follows.

Condensed protocol. Cat 302; female; weight 1.65 kgm.

Left pupil contracted and nictitating membrane forward. Left adrenal denervated and left superior cervical ganglion excised two weeks previously.

- 11.15 a.m. Injected 5 mgm. pilocarpine hydrochloride subcutaneously.
- 11.20 a.m. Salivation; no change in pupils.
- 11.25 a.m. Injected 5 mgm. pilocarpine hydrochloride.
- 11.30 a.m. Vomited; both pupils dilated widely, the left more than the right; both nictitating membranes retracted (remained retracted throughout the rest of the experiment). Considerable salivation.
- 11.40 a.m. Right pupil $\frac{2}{3}$ to $\frac{3}{4}$ dilated, left pupil dilated to maximal.
- 12.50 p.m. Pupils equal (about $\frac{3}{4}$ dilated).
- 1.19 p.m. Injected 5 mgm. pilocarpine hydrochloride.
- 1.23 p.m. Vomited; same effect on pupils as noted in observation of 11.30.
- 2.40 p.m. Pupils equal (about $\frac{2}{3}$ dilated). Injected 6 mgm. pilocarpine hydrochloride.
- 3.05 p.m. Vomited; transitory dilatation of both pupils, the left becoming wider than right, returning to equality in a few minutes; salivation and lachrymation pronounced.
- 4.00 p.m. Injected 10 mgm. pilocarpine hydrochloride. The injection caused the cat to become violently angry, and bit its attendant; during this rage both pupils dilated widely the left becoming maximal and the right nearly so. As soon as the cat became pacified the pupils returned to equality.

4.50 p.m. Pupils equal (about $\frac{3}{4}$ dilated) and nictitating membranes still retracted. The cat was now killed by a sudden blow on the head.

The left adrenal weighed 0.25 gram and contained 0.31 mgm. epinephrin. The right adrenal weighed 0.25 gram and contained 0.24 mgm. epinephrin.

There was no marked depletion of the epinephrin store in the unprotected (right) gland as compared with the denervated (left) gland. There was, however, a small but definite difference between the two glands (0.31 mgm. in the left, and 0.24 mgm. in the right). Elliott (13) reported that he failed to find proof that pilocarpine influences the adrenalin load in any way. He quotes two experiments in which the pilocarpine was administered to cats under ether. In the first experiment the protected gland contained 0.26 mgm. epinephrin and the other 0.17 mgm. In the second experiment the protected gland contained 0.30 mgm. epinephrin and the other 0.24 mgm. (assayed by the blood pressure method). In Elliott's experiments it would be impossible to separate the effect of ether on the epinephrin store from that of the pilocarpine. For he has shown, and we have confirmed his observation (14) that ether causes a diminution in the store. In our experiment the cat was not anesthetized, the drug being administered subcutaneously. The effect produced by pilocarpine on the store is apparently much less than that caused by some other drugs (morphine, β -tetra-hydranaphthylamine).

Our experiments with pilocarpine indicate that the rate of output of epinephrin from the adrenals is not materially altered under the influence of this drug. In only one experiment (cat 287) was the output apparently increased to about double the initial rate before pilocarpine was injected, and this increase only brought the output up to the ordinary average rate found by us in the absence of influence of drugs, the initial rate being somewhat lower than the average. The only other evidence of a possible action exerted by this drug was obtained in cat 289. The high epinephrin concentration found in the fourth specimen (blood flow 0.52 gram per minute) may indicate that, under certain

conditions, pilocarpine is probably capable of increasing the concentration of epinephrin in adrenal vein blood beyond the normal possible maximum usually found in specimens collected with very slow blood flows. Whatever action, if there is any genuine action on the epinephrin secretion from the adrenals, that is caused by pilocarpine, it is certainly not comparable to the pronounced effect caused by this drug on the secretion of saliva, sweat, etc.

SUMMARY

1. The rate of output of epinephrin from the adrenals (in cats) is not materially influenced by the action of atropine or pilocarpine. A moderate increase in the rate of liberation may be produced by atropine, which in large doses may be preceded by a moderate transient depression of the rate of output. The augmentation of the output, if any is caused, by pilocarpine is small and is not comparable with the large increase in the rate of epinephrin liberation caused by strychnine or the immediate effect of nicotine.

2. One experiment was performed which indicates that pilocarpine is capable of producing a moderate depletion of the epinephrin store, in adrenal glands with intact nerve supply.

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FURTHER OBSERVATIONS ON THE RELATION OF THE SPINAL CORD TO THE SPONTANEOUS LIBERATION OF EPINEPHRIN FROM THE ADRENALS, AND THE ACTION OF STRYCHNINE AFTER CERVICAL CORD SECTION

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In a previous paper (1) we concluded that in the cat after transection of the spinal cord in the cervical region the epinephrin output is not abolished, but is still sustained from the upper thoracic cord. In acute experiments it was shown that the previous rate of output might continue unchanged after the cervical cord transection, indicating that the mechanism in the thoracic cord was actually functioning at the moment of the cervical section. It is obvious that if there is an epinephrin-secretory center in the thoracic cord it may be subject to spinal shock after lesions of the cervical cord like other spinal centers, and that the susceptibility to shock might be expected to vary in different kinds of animals and might be influenced by the experimental conditions, especially the circulatory changes caused by the cervical lesion. In the present research, accordingly, we have employed not only cats, but also dogs and one or two monkeys and rabbits, and have eliminated the upper portions of the central nervous system in cats by ligation of their arterial supply as well as by the knife. The stimulating action of strychnine upon the central mechanisms governing the epinephrin output having become known to us since our last paper, we have made a number of experiments in which this drug was used after the cervical transection, in order to see whether the epinephrin output was increased by it, as is the case when the nervous system is intact and the epinephrin being liberated at the ordinary rate.

Note. By "ordinary output" or "normal output" we mean an output within the range observed by us in animals in which we have not purposely used procedures calculated to alter the output (such as the administration of drugs like

strychnine or artificial stimulation of the splanchnic nerves). In referring to this normal output we often use the phrase "under our experimental conditions," or words to that effect. If we sometimes omit this qualifying phrase it is simply because its continual repetition becomes tedious. It is quite true that as we have gone on and have seen within how relatively narrow a range the output, as measured by us, varies with different anesthetics and different operations, we have inevitably tended more to the opinion that a quantity which is not demonstrably affected by changes in such important experimental conditions must depend upon something more fundamental than the anesthesia, opening of the abdomen, etc. The fact that the output in one and the same acute experiment is sensibly the same when adrenal blood is first obtained as it is later on when additional operative procedures have been employed, suggests that what we term the ordinary or normal spontaneous output is not initiated or sustained by the trauma. Cannon (2) has affirmed that there is no doubt that opening the abdominal cavity under anesthesia results in a discharge of impulses along the splanchnic fibers governing the adrenal secretion. "The adrenal glands, therefore, are continuously and abnormally stimulated if the abdomen is opened." The only evidence he offers for this is the statement that opening the abdomen causes impulses to descend the splanchnic fibers to the intestines which inhibit their movements. As the adrenal secretory fibers are also sympathetic and run in the splanchnics they must be stimulated too. It would be just as logical to say that as vasoconstrictor fibers for the splanchnic area are sympathetic and run in the splanchnics they must also be stimulated and therefore there should be a marked rise of blood pressure due to splanchnic vasoconstriction on opening the abdomen. The only way of showing that opening the abdomen increases the epinephrin output is to prove it experimentally and this has not been done. The same is true of the alleged influence of anesthetics. Cannon says "evidence exists in the inhibitory influence of anesthesia on gastro-intestinal movements that anesthesia alone can arouse splanchnic impulses." And among these he obviously includes impulses passing along the splanchnics to the adrenals to increase the epinephrin output. Yet he has stated in another paper (3) that he has found anesthesia by urethane to be "in fact unattended by any increase of adrenal secretion demonstrable by our tests." Now if urethane does not increase the output, ether and morphine certainly cannot do so since we have found the output with these anesthetics less if anything on the average than with urethane. While we do not think that Cannon's work has shown anything at all as to the influence or want of influence of urethane upon the epinephrin output, and he has published no experimental data whatever upon this point, his conclusion that the employment of an anesthetic has no influence upon the results of his own experiments whereas the employment of the same anesthetic vitiates ours, is somewhat puzzling. But not only can he use urethane with impunity, while he indicates that we cannot do so, but he can open the abdomen, of course under anesthesia, and still obtain a certain reaction with asphyxia which he interprets as proving an increased output of epinephrin, whereas he says that when we open the abdomen we immediately deprive ourselves of all possibility of obtaining evidence of any such effect since we are working with an animal under an anesthetic and with opened abdomen. Cannon's suggestion that the reason we fail to obtain evidence of an increased output of epinephrin in such conditions as asphyxia is that the opening of the abdomen produces a secretion which

is "unsurpassable." This suggestion is inconsistent with his own statements that the output of epinephrin caused by stimulation of the central end of the sciatic is "from 5 to 25 times the amount regarded by Stewart and Rogoff as the normal output," and that the reaction on which he relies to demonstrate the increased output is elicited in equal intensity by asphyxia.

The results of the greater part of the experiments are condensed into three tables. Only a sufficient number of protocols to illustrate the various types of experiment or to illustrate experiments which could not be tabulated have been introduced.

ACUTE EXPERIMENTS ON CATS

In table 1 are shown the results of acute experiments on cats in which the epinephrin output was assayed on rabbit intestine (and uterus) segments before and after transection of the cervical cord.

In three of the cats (350, 407 and 67) the output, which was of the ordinary magnitude before the cord section, remained practically unaltered by that operation. The data on one of these animals have already been published (1), but for the sake of completeness they are included in the table. In one of these cats (407) the intravenous injection of strychnine after transection of the cord raised the output of epinephrin to seven times the initial value. As the experiment illustrates at the same time the possibility of dividing the cervical cord without reducing the epinephrin output and without interfering with the characteristic action of strychnine upon the output, the condensed protocol is reproduced.

Condensed protocol. Cat 407; female; weight, 2.46 kgm.

- 9.05 a.m. Anesthetized with ether, inserted cannulae into trachea, carotid artery and external jugular vein. Obtained a specimen of indifferent (jugular) blood.
- 9.20 a.m. Exposed cord below mid-cervical region. Made cava pocket, tying coeliac axis, renal and mesenteric arteries and abdominal aorta (above bifurcation) in addition to the veins entering the cava pocket.
- 9.45 a.m. Cava pocket completed. Collected adrenal blood.
- 9.48½ a.m. First specimen, 1.9 gram in 30 seconds (3.8 grams per minute).
- 9.49 a.m. Second specimen, 4.85 grams in 90 seconds (3.2 grams per minute).
- 9.55 a.m. Blood pressure 103 mm. of mercury.
- 10.00 a.m. Transected cord above the origins of the 6th pair of cervical nerves (position of section was verified at autopsy). Spontaneous respiration continued; artificial respiration not employed. After cord section the pupils were about one-third dilated and eye reflexes good.

- 10.03 a.m. Blood pressure 50 mm. of mercury.
10.05 a.m. Third adrenal specimen, collected for 30 seconds (not weighed).
10.05½ a.m. Fourth adrenal specimen, 1.85 gram in 5 minutes (0.37 gram per minute).
10.10 a.m. Started artificial respiration.
10.15 a.m. Blood pressure 50 mm. of mercury.
10.17 a.m. Intravenous injection of 0.45 mgm. strychnine: within ½ minute after strychnine injection a tonic spasm occurred for a few seconds and this was followed by clonic convulsions which recurred intermittently during the rest of the experiment.
10.20 a.m. Fifth adrenal specimen, collected for 30 seconds (not weighed).
10.20½ a.m. Sixth adrenal specimen, 2.3 grams in 10 minutes (0.23 gram per minute).
10.33 a.m. Blood pressure 30 mm. of mercury. Another specimen of indifferent blood was now obtained. Combined weight of adrenals, 0.375 gram. (There was about 75 per cent of serum in the bloods).

As it is impossible to reproduce all of the tracings used for the assay even in one experiment, we shall illustrate this one typical experiment more completely than the rest, for which it must serve as a sample of the manner in which we arrive at the epinephrin concentrations. Some of the tracings are reproduced in figures 1 to 4.

The 2nd specimen was found to be decidedly weaker than 1:4,000,000 adrenalin (fig. 1, confirmed by other observations), decidedly stronger than 1:8,000,000, somewhat stronger than 1:6,660,000, somewhat weaker than 1:5,300,000. It was taken at 1:6,000,000, corresponding to an output of 0.00053 mgm. per minute for the cat, or 0.00021 mgm. per kgm. per minute.

The 4th specimen, collected after section of the cord, was much stronger than 1:1,300,000 (fig. 2), not very different from 1:660,000 (fig. 3). Another observation indicated that it was probably somewhat weaker than 1:660,000. It was distinctly weaker than 1:530,000 (fig. 3). It was taken at 1:700,000, corresponding to an output of 0.00053 mgm. per minute for the cat, or 0.00021 mgm. per kgm. per minute.

The concentration of epinephrin in the 6th adrenal specimen, obtained after injection of strychnine, was so great that in order to make a good assay it was necessary to dilute it with indifferent blood. In this way it was shown to be much stronger than 1:165,000 adrenalin (fig. 4). The 6th specimen diluted with 3 volumes of indifferent blood was much stronger than 1:660,000 adrenalin. Diluted with 7 volumes of indifferent blood it was still found to be much stronger than 1:1,000,000, i.e., the 6th specimen was much stronger than 1:125,000.

TABLE 1
Acute experiments. Cats

NUMBER OF ANIMAL	BEFORE SECTION OF CERVICAL CORD							AFTER SECTION OF CERVICAL CORD							REMARKS			
	Body weight	Level of cord section	Combined weight of adrenals	Blood collected	Duration of collection	Blood flow per minute	Blood pressure	Epinephrin concentration	Epinephrin output per minute		Blood flow per minute	Blood pressure	Epinephrin concentration	Epinephrin output per minute				
									For animal	Per kilogram				For animal		Per kilogram		
320	3.15	v-vi	0.295	3.25	300	0.65	40	1:6,000,000	0.00011	0.000035	4.4	330	0.8	44	1:6,000,000	0.00013	0.00004	Shock on cord exposure: 80 cc. Ringer injected before collection of each adrenal specimen.
321	3.89	above iv	0.922	2.75	360	0.46		1:2,700,000	0.00017	0.000045	2.05	720	0.17		1:1,000,000	0.00017	0.000045	Without injection of Ringer
											2.55	360	0.425		1:2,700,000	0.00016	0.00004	With Ringer injection, 100 cc.
322	2.25	v-vi	0.487	7.0	120	3.3	144	1:4,500,000	0.0007	0.0003	3.4	720	0.30	58	1:800,000	0.0004	0.00017	
											1.3	600	0.13	37	1:500,000	0.00026	0.00011	
384	2.63	above vi	0.275	4.95	120	2.5	117	1:3,500,000	0.0007	0.00027	7.1	90	4.7	46	1:9,400,000	0.0005	0.0002	After cord section the blood pressure fell to 32 mm. of mercury. 100 cc. Ringer injected and circulation improved.

399	2.69	v-vi	0.268	5.3	903.5	110	1:5,000,000	0.00065	0.00025	1.5	330	0.3	47	1:1,500,000	0.0002	0.00075	0.5 mgm. strychnine injected before collection of this specimen.
										1.8	240	0.45	40	1:250,000	0.0018	0.0007	
406	2.09	vi-vii	0.474	3.75	1201.87	112	1:4,600,000	0.0004	0.00024	3.15	120	1.57	86	1:6,000,000	0.00025	0.00012	After cord section blood pressure fell to 26 mm. 60 cc. Ringer injected and blood pressure rose to 86 mm. of mercury.
										1.15	360	0.2	40	1:150,000	0.0013	0.0006	0.6 mgm. strychnine injected before this specimen.
407	2.46	above vi	0.375	4.85	903.2	103	1:6,000,000	0.00053	0.00021	1.85	300	0.37	50	1:700,000	0.00053	0.00021	0.45mgm. strychnine injected before this specimen.
										2.3	600	0.23	30	1:70,000	0.0033	0.0014	
418	1.96	v	0.232	(a)3.2 (b)3.35	902.1 1201.7	105 94	1:4,000,000 1:1,700,000	0.0005 0.001	0.00025 0.0005	1.0	600	0.1	24	1:100,000	0.001	0.0005	0.25 mgm. strychnine injected after (a). Blood pressure rose to 120 mm. Hg. Cord section after strychnine injection.
67	2.31	above v	0.440	10.0	1205.0		1:13,000,000	0.0004	0.00017	2.1	430	0.3	1	800,000	0.0004	0.00017	

TABLE 1—*Concluded*

NUMBER OF ANIMAL	BEFORE SECTION OF CERVICAL CORD										AFTER SECTION OF CERVICAL CORD						REMARKS	
	Body weight	Level of cord section	Combined weight of adrenals	Blood col-lected	Duration of collection	Blood flow per minute	Blood pres-sure	Epinephrin concentration	Epinephrin output per minute		Blood col-lection	Duration of collection	Blood flow per minute	Blood pres-sure	Epinephrin concentration	Epinephrin output per minute		
									For animal	Per kilogram						For animal		Per kilogram
324	2.1		0.367	5.55	180	1.85		1:9,000,000	0.0002	0.0001	4.75	300	0.95	1:4,000,000	0.00024	0.0001	Bulb and brain eliminated by ligation of head arteries.	
32	2.05		0.252	5.15	180	1.72		1:2,500,000	0.0007	0.00035	2.3	300	0.5	1:675,000	0.0007	0.00035	Bulb and brain eliminated by ligation of head arteries.	
435	2.5		0.314	1.9	150	0.76		1:800,000	0.00095	0.00038	2.5	180	0.83	1:700,000	0.0012	0.00048	Bulb and brain eliminated by ligation of head arteries.	
											1.65	360	0.275	1:450,000	0.00061	0.00024		
383*	3.98	v-vi	0.602	8.05	90	5.4	129	1:5,400,000	0.001	0.00025	4.45	180	1.48	93	1:3,000,000	0.0005	0.00013	Both adrenals discharging in all specimens.
385*	2.75	iv	0.612	6.2	180	2.06	114	1:2,900,000	0.0007	0.00025	3.25	240	0.81	40	1:1,000,000	0.0008	0.0003	Right adrenal only discharging in both specimens.

* In these two animals the spinal operation was a semisection, left in 383, and right in 385.

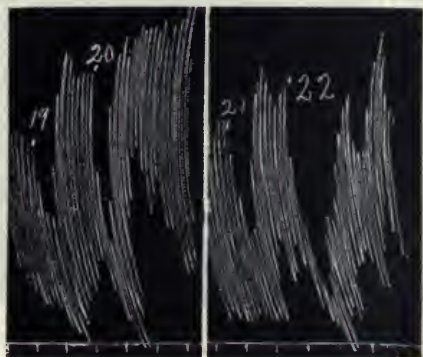


Fig. 1. Intestine tracings. Bloods from cat 407. At 19 and 21 Ringer was replaced by indifferent (venous) blood and this at 20 by the 2nd adrenal specimen (collected before cervical cord section); at 22 by indifferent blood to which was added adrenalin to make a concentration of 1:4,000,000. All the bloods were diluted with 3 volumes Ringer (the adrenalin blood after adding the adrenalin). As in all the figures the time trace is in half-minutes. Reduced to one-half.

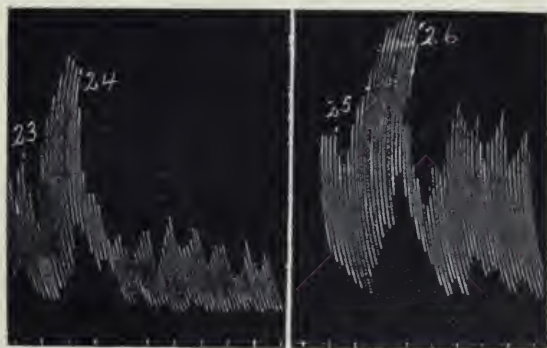


Fig. 2. Intestine tracings. Bloods from cat 407. At 23 and 25 Ringer was replaced by indifferent (venous) blood and this at 24 by the 4th adrenal specimen (collected 5½ minutes after cervical cord section); at 26 by indifferent blood to which was added adrenalin to make a concentration of 1:1,300,000. All the bloods were diluted with 3 volumes Ringer (the adrenalin blood after adding the adrenalin). Reduced to three-sevenths.

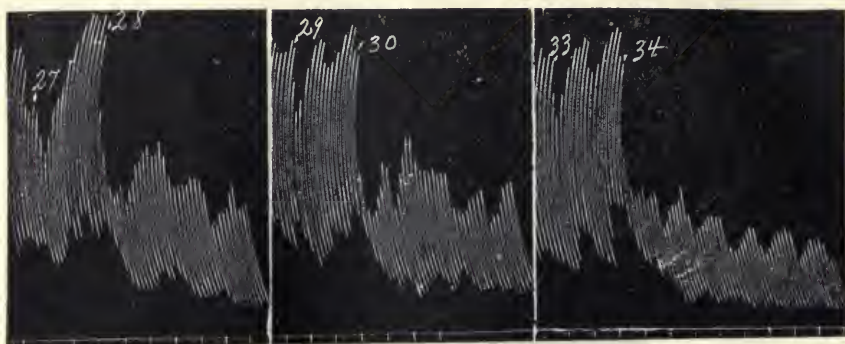


Fig. 3. Intestine tracings. Bloods from cat 407. At 27, 29 and 33 Ringer was replaced by indifferent (venous) blood and this at 28 by indifferent blood to which was added adrenalin to make a concentration of 1:660,000; at 30 by the 4th adrenal specimen (collected 5½ minutes after cervical cord section); at 34 by indifferent blood to which was added adrenalin to make a concentration of 1:530,000. All the bloods were diluted with 3 volumes Ringer (the adrenalin bloods after adding the adrenalin). Reduced to three-sevenths.

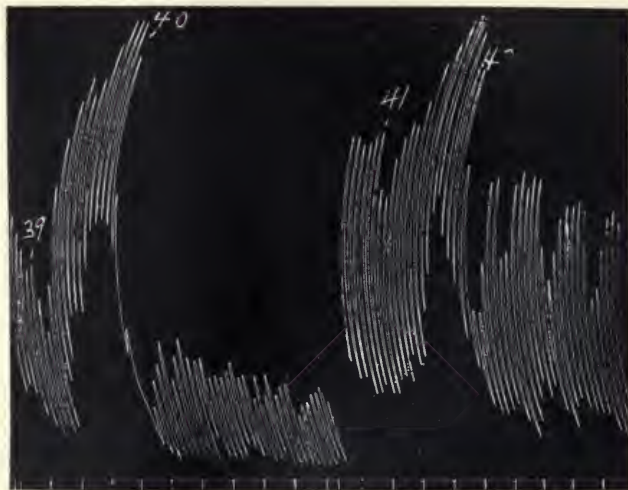


Fig. 4. Intestine tracings. Bloods from cat 407. At 39 and 41 Ringer was replaced by indifferent (arterial) blood and this at 40 by the 6th adrenal specimen (collected 3 minutes after intravenous injection of strychnine and 10 minutes after cervical cord section) diluted with 3 volumes of indifferent blood; at 42 by indifferent blood to which was added adrenalin to make a concentration of 1:660,000. All the bloods were diluted with 3 volumes Ringer (the adrenalin blood after adding the adrenalin). Reduced to one-half.

In the same way it was shown that the specimen did not differ much from 1:66,000, being probably a little weaker. This was confirmed by numerous observations on the adrenal blood diluted with 7 volumes of indifferent blood, and also on the adrenal blood diluted with 14 volumes of indifferent blood. The 6th specimen was finally taken at 1:70,000, corresponding to an output of 0.0033 mgm. per minute for the cat, or 0.0014 mgm. per kgm. per minute.

In two of the cats in table 1 (320 and 321) the output was already much below the ordinary average before the cord was cut. These were two of the earliest experiments, and at this time we were in the habit of passing a thread under the cord after exposure. The thread was not tied, of course, but was used to make sure that the transection was complete. Later on the section was made intradurally through a longitudinal slit in the dura by means of small blunt pointed scissors, and the cord was not disturbed before collection of the initial adrenal specimen, apart from being laid bare at the level where it was to be divided. Blood vessels lying between the cord and the walls of the vertebral canal escaped injury except, of course, where the bone was removed. In cats 320 and 321 the low initial output of epinephrin was associated with a low blood pressure. Transection of the cord left the output unaltered. The condensed protocol of cat 321 follows.

Condensed protocol. Cat 321; male; weight, 3.89 kgm.

- 8.30 a.m. Urethane, 6 grams per stomach tube.
- 9.00 a.m. Inserted tracheal cannula, exposed cord in mid-cervical region and placed a loose ligature under the cord. During the manipulations around the cord the cat stopped breathing and the circulation became feeble. Artificial respiration was at once started. Obtained a specimen of indifferent blood (femoral vein). Made cava pocket.
- 10.06 a.m. Pocket completed. The coeliac axis, renal and mesenteric arteries and abdominal aorta (above the bifurcation) were tied in addition to the veins entering the cava pocket. Collected adrenal blood.
- 10.10 a.m. First specimen, 0.9 gram in 1 minute.
- 10.11 a.m. Second specimen, 2.75 grams in 6 minutes (0.46 gram per minute).
- 10.20 a.m. Transected cord just above the origins of the 4th pair of cervical nerves.
- 10.23 a.m. Third adrenal specimen collected for 1 minute (not weighed).
- 10.24 a.m. Fourth adrenal specimen, 2.05 grams in 12 minutes (0.17 gram per minute).
- 10.35 a.m. Intravenous injection of 50 cc. Ringer.
- 10.45 a.m. Intravenous injection of 50 cc. Ringer.
- 10.50 a.m. Fifth adrenal specimen, collected for 1 minute.
- 10.51 a.m. Sixth adrenal specimen, 2.55 grams in 6 minutes (0.425 gram per minute). Another specimen of indifferent blood was now obtained from the right heart. Combined weight of adrenals 0.922 gram.

We have had many illustrations in this work of a fact which may have some general bearing upon the mechanism of spinal shock but whose bearing upon that depression of the spinal mechanism concerned in the liberation of epinephrin sometimes observed after cervical cord transection, and which we suggest is a spinal shock phenomenon, seems incontestable. When the blood pressure and therefore the blood flow through the adrenals are lowered very considerably while the central nervous system is intact, we always find a corresponding increase in the epinephrin concentration in the adrenal vein blood, which until the possible maximum concentration has been attained keeps the output of epinephrin per unit of time as high as the initial output, at any rate for the relatively short duration of the low blood pressure which we have studied. That is to say, with intact nervous system the epinephrin secreting mechanism in the thoracic cord continues to sustain the original output in spite of the impaired circulation. It is, of course, to be supposed that after the low blood pressure has endured for a considerable time (vascular shock) the output will be diminished. But this point is not easily reached in experiments of moderate duration. When, however, injury to the cervical cord is associated with a low blood pressure, which would not of itself cause diminution of the epinephrin output, the output may be markedly diminished and then a slow adrenal blood flow may be accompanied by a great *decline* in the concentration of epinephrin in the adrenal vein blood.

It has often been pointed out that spinal shock cannot be due to the fall of blood pressure since this affects equally the parts of the central nervous system above and below the lesion. No doubt in a general way this is true enough. But if attention be paid to the fact that the direction of flow of the blood in the main spinal arteries is caudad and the direction of flow of a good deal of the venous blood cephalad, it may appear that for some distance below a spinal transection, especially perhaps in the cervical region, the possibility would exist of a greater interference with the circulation below the lesion, for a given fall of blood pressure, than above it, in spite of the lateral anastomoses. In any case, it seems to follow pretty clearly from our observations on cats that the rupture of the upper paths is more likely to cripple the epinephrin secretory mechanism in the thoracic cord if accompanied by an unusually low blood pressure than when the circulation remains relatively efficient after the cervical cord lesion. The loss of impulses from above is the same in both cases, but the well nourished thoracic mechanism goes on sustaining the epinephrin output at a high or at the

original level, whereas the poorly nourished mechanism in the absence of those impulses is unable to do this.

In cats 322, 399, 406 (table 1) the output was diminished after transection of the cord to one-half to one-third of the initial output. When strychnine was given, in two of the animals in which the output had been diminished by the cord section, it was raised not only to the initial value but to three times that amount. Included in table 1 are three experiments in which the upper parts of the central nervous system were eliminated by permanent ligation of the innominate, left subclavian and other arteries supplying the head with blood. There could not be any doubt that at the time of collection of the adrenal blood after ligation of the arteries the elimination of the bulb and everything above it was complete. The eye reflexes had long since disappeared, the respiratory center had ceased to discharge, the swallowing center had become inactive, and so on. The observations of Stewart, Guthrie, Burns and Pike (4) show that after such ligation of arteries in cats there is total elimination of the bulb and everything above it and some interference with upper cervical segments.

In these animals, without exception, the epinephrin output after elimination of the bulb and everything above it by the bloodless method was not at all diminished. It is clear that by this method the infliction of direct injury upon the portion of the cord concerned in sustaining the epinephrin output by such gross interference with its circulation as might be caused by subdural hemorrhage, cutting and blocking of spinal arteries and veins, is largely obviated. The residual blood pressure after elimination of the brain and bulb is probably also in general somewhat higher than when the cervical cord is divided by the knife, and the proportion of experiments in which the output continued without diminution is increased. Two of the protocols follow.

Condensed protocol. Cat 325; male; weight, 2.05 kgm.

- 9.20 a.m. to 9.50 a.m. Anesthetized with ether, inserted tracheal cannula, placed ligatures around the innominate, left subclavian and both carotid arteries, but did not tie off yet, started artificial respiration (pleura punctured).
- 10.10 a.m. Cava pocket completed; coeliac axis, renal, mesenteric arteries and abdominal aorta (above bifurcation) tied in addition to the veins entering the cava pocket. Collected adrenal blood.
- 10.14 a.m. First specimen, 1.2 gram in 30 seconds (2.4 grams per minute).
- 10.14½ a.m. Second specimen, 5.15 grams in 3 minutes (1.72 gram per minute).
- 10.22 a.m. Tying off head arteries completed.

- 10.23 a.m. Pupils maximally dilated. No eye reflexes present. Gums pale. Intra-ocular pressure diminished. No spontaneous respiratory movements.
- 10.26½ a.m. Third adrenal specimen, 0.6 gram in 30 seconds (1.2 gram per minute).
- 10.27 a.m. Fourth adrenal specimen, 2.3 grams in 5 minutes (0.5 gram per minute).
- 10.32 a.m. No change in symptoms showing complete cerebral anemia and no change observed thereafter throughout the rest of the experiment. Obtained (indifferent) venous blood. Combined weight of adrenals, 0.252 gram.
- Autopsy.* All of the head arteries were tied off (i.e., innominate, left subclavian, proximal to the vertebrae and both carotids).

The 6th specimen was shown to have a higher concentration than the 4th. As the concentration of the 4th specimen was 1:675,000, the 6th must have had a maximal concentration, although of course with the slow flow a full calculated output could not be expected.

Condensed protocol. Cat 435; female (pregnant); weight 2.5 kgm.

- 10.15 to 10.45 a.m. Anesthetized with ether, inserted tracheal cannula, placed ligatures around the innominate, left subclavian (proximal to origin of vertebral), and both carotid arteries, but did not tie them off.
- 11.00 a.m. Cava pocket completed. The coeliac axis, renal and mesenteric arteries and abdominal aorta (above bifurcation) tied, in addition to the veins entering the cava pocket. Collected adrenal blood.
- 11.04 a.m. First specimen, 0.6 gram in 30 seconds (1.2 gram per minute).
- 11.04½ a.m. Second specimen, 1.9 gram in 2½ minutes (0.76 gram per minute).
- 11.08 a.m. Cat lightly anesthetized; pupils contracted; eye reflexes good; started tying cerebral vessels.
- 11.10 a.m. Head arteries tied. Pupils widely dilated and eye reflexes gone. Cat still gasping.
- 11.12 a.m. Mucosa of mouth and nose pale. Cat still gasps occasionally.
- 11.14 a.m. Hemostat clamped on left subclavian proximal to ligature.
- 11.15 a.m. No gasps; all upper reflexes gone, tail and hind leg reflexes still present, notwithstanding ligation of vessels in making cava pocket; intra-ocular tension low; swallowing reflex gone. A nick in nasal septum does not bleed.
- 11.25 a.m. Third adrenal specimen, 0.8 gram in 30 seconds (1.6 gram per minute).
- 11.25½ a.m. Fourth adrenal specimen, 2.5 grams in 3 minutes (0.83 gram per minute).
- 11.38 a.m. All symptoms of complete cerebral anemia still the same.
- 11.40 a.m. Fifth adrenal specimen, collected for 30 seconds.
- 11.40½ a.m. Sixth adrenal specimen, 1.65 gram in 6 minutes (0.275 gram per minute). Now tied off the adrenal veins and obtained (indifferent) venous blood from the cava. Combined weight of adrenals 0.314 gram.

Autopsy: Verified the complete ligation of all blood vessels going to the head.

In cat 435 the 2nd adrenal specimen, taken before ligation of the head arteries was somewhat weaker than the 4th specimen, collected 15 minutes after completion of the ligation (fig. 5, confirmed by other observations). Diluted with 3 volumes of indifferent blood it was shown to be somewhat stronger than 1:3,600,000 adrenalin, i.e., the 2nd specimen was somewhat stronger than 1:900,000. It was finally assayed at 1:800,000, corresponding to an output of 0.00095 mgm. per minute for the cat, or 0.00038 mgm. per kgm. per minute.

The 4th specimen was proved to be much stronger than 1:1,350,000, stronger than 1:900,000. Diluted with 3 volumes of indifferent blood



Fig. 5. Intestine tracings. Bloods from cat 435. At 1 and 3 Ringer was replaced by indifferent (venous) blood and this at 2 by the 2nd adrenal specimen (collected before tying off head arteries); at 4 by the 4th adrenal specimen (collected after tying off head arteries). All the bloods were diluted with 3 volumes Ringer. Reduced to one-half.

it was not much different from 1:2,700,000, i.e., the 4th specimen was about the same as 1:675,000. Taking it at 1:700,000 we get 0.0012 mgm. per minute as the output for the cat, or 0.00048 mgm. per kgm. per minute. In other words, the complete elimination of the bulb and brain had left the output fully as great as the initial output.

The 6th adrenal specimen, collected 15 to 20 minutes after the end of collection of the 4th specimen, that is after a period of more than half an hour of complete cerebral anemia, was diluted with 3 volumes of indifferent blood before assay. So diluted it was found to differ little in con-

centration from 1:1,800,000 adrenalin, being if anything a little stronger i.e., the 6th specimen was equivalent to about 1:450,000 adrenalin. It was decidedly weaker than 1:325,000. Taking it at 1:450,000 we get an output of 0.00061 mgm. per minute for the cat, or 0.00024 mgm. per kgm. per minute. The high concentration shows that at this time the adrenal secretion was still quite efficient in the absence of the bulb and brain. With the slow blood flow it could not of course be expected that a full output could be calculated out from such a concentration.

Two experiments are included in table 1 in which the cervical cord was semisected (cats 383 and 385). In one of these (383) after the semisection the output was reduced to about one-half the initial volume. This cannot be interpreted as indicating that the semisection diminished or abolished the output from the corresponding adrenal, but rather that the cord lesion caused a depression in the output from the two adrenals (spinal shock). For in the other cat (385), division of the right half of the cord did not interfere at all with the output of epinephrin from the right adrenal. In this cat the left adrenal was clipped off during collection of the blood, both before and after the semisection.

In addition to the cats included in table 1 in which segment assays were made of the adrenal blood, the epinephrin output was estimated by the reaction upon the pupil (sensitized by previous removal of the superior cervical ganglion) in two more cats (350 and 353). The condensed protocols follow.

Condensed protocol. Cat 350; male, weight 1.82 kgm.

Left superior cervical ganglion excised 13 days prior to experiment. Left pupil contracted and nictitating membrane forward.

9.00 a.m. Urethane, 4 grams by stomach tube.

10.00 a.m. Left pupil $\frac{2}{3}$, right pupil $\frac{1}{2}$ maximal, both nictitating membranes retracted.

10.00 to 10.20 a.m. Inserted tracheal cannula and exposed cord in mid-cervical region. On exposing cord the left pupil became maximally dilated, the right pupil remained slit-like, both nictitating membranes were retracted. In a few minutes the left pupil came down to $\frac{1}{2}$ maximal dilatation.

10.22 a.m. Started artificial respiration and made "long" pocket, tying the coeliac axis, renal and mesenteric arteries and abdominal aorta (above bifurcation) in addition to the veins entering the cava pocket.

10.40 a.m. Pocket completed. Right pupil slit-like, left pupil $\frac{1}{2}$ dilated, both nictitating membranes slightly forward, the right more than the left.

- 10.52 to 11.08 a.m. Two observations showed that a 2-minute pocket was equal to 1.0 cc. of 1:1,500,000 adrenalin, corresponding to an output of 0.00035 mgm. per minute for the cat, or 0.0002 mgm. per kgm. per minute.
- 11.15 a.m. Transected cord through the origins of the 5th pair of cervical nerves (position of cord section was verified at autopsy).
- 11.18 to 11.26 a.m. Two pocket observations showed a 2-minute pocket to correspond to 1.0 cc. of 1:1,500,000 adrenal, i.e., the output was the same as before cord section. Combined weight of adrenals, 0.41 gram.

Condensed protocol. Cat 353; old male; weight, 3.13 kgm.

Left superior cervical ganglion excised 19 days prior to experiment. Left pupil contracted and nictitating membrane forward.

- 9.15 a.m. Urethane, 4 grams by stomach tube.
- 10.20 a.m. Urethane, 2 grams by stomach tube.
- 10.45 a.m. Exposed cord in mid-cervical and mid-dorsal regions. Loose ligatures passed under cord.
- 11.20 a.m. Cava pocket completed. The coeliac axis, renal and mesenteric arteries and abdominal aorta (above the bifurcation) were tied in addition to the veins entering the cava pocket.
- 11.22 a.m. Right pupil slit-like, left pupil $\frac{1}{2}$ dilated, both nictitating membranes forward.
- 11.23 to 11.41 a.m. Three observations showed that a 2-minute pocket was equal to 0.5 cc. of 1:660,000 adrenalin, corresponding to an output of 0.00037 mgm. per minute for the cat, or 0.00012 mgm. per kgm. per minute.
- 11.45 a.m. Transected cord just above the origins of the 5th pair of cervical nerves.
- 11.48 a.m. to 12.00 m. Three observations showed that a 2-minute pocket was fully equivalent to 0.5 cc. of 1:1,000,000 adrenalin, corresponding to an output of fully 0.00025 mgm. per minute for the cat, or 0.00008 mgm. per kgm. per minute. Combined weight of adrenals, 0.705 gram.

ACTION OF STRYCHNINE AFTER CERVICAL CORD TRANSECTION

As already stated, in a number of the cats strychnine was injected after the cervical transection in order to see whether the stimulating action exerted by it upon the epinephrin output in animals with intact cord would be developed after elimination of the upper parts of the central nervous system. This was found to be the case. Increases in the output both relatively and absolutely as great as those seen with intact cord were obtained. In one of the experiments already discussed (cat 407) the initial output remained unchanged after the cord

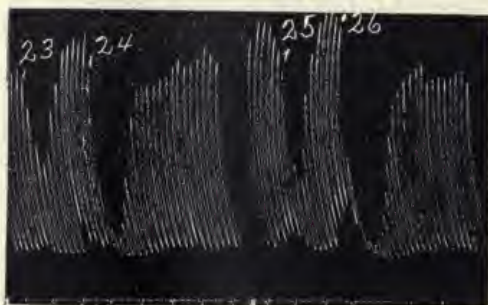


Fig. 6. Intestine tracings. Bloods from cat 406. At 23 and 25 Ringer was replaced by indifferent blood and this at 24 by the 4th adrenal specimen (collected 14 minutes after cervical cord section) and at 26 by indifferent blood to which was added adrenalin to make a concentration of 1:4,000,000. All the bloods were diluted with 3 volumes Ringer (the adrenalin blood after adding the adrenalin). Reduced to one-half.

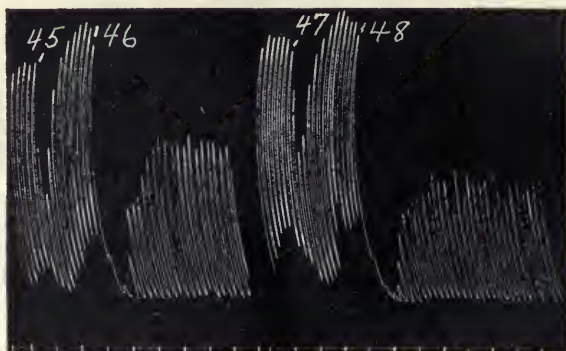


Fig. 7. Intestine tracings. Bloods from cat 406. At 45 and 47 Ringer was replaced by indifferent (venous) blood and this at 46 by indifferent blood to which was added adrenalin to make a concentration of 1:1,330,000, and at 48 by the 6th adrenal specimen (collected 5 minutes after intravenous injection of strychnine and 33 minutes after cervical cord section) diluted with 4 volumes of indifferent blood. All the bloods were diluted with 3 volumes Ringer (the adrenalin blood after adding the adrenalin). Reduced to one-half.

section and was increased sevenfold by strychnine. In another experiment (cat 406) the output was diminished to something more than half the original output by the cervical transection and was brought up to three times the initial output by strychnine.

Figures 6 to 8 illustrate the assay in cat 406. The 2nd adrenal specimen was found to be much stronger than 1:5,300,000, somewhat stronger than 1:4,000,000 (confirmed by a number of observations), weaker than 1:2,750,000 (confirmed by a number of observations). It was finally taken at 1:3,600,000, corresponding to an output of 0.0005 mgm. per minute for the cat, or 0.00024 mgm. per kgm. per minute.

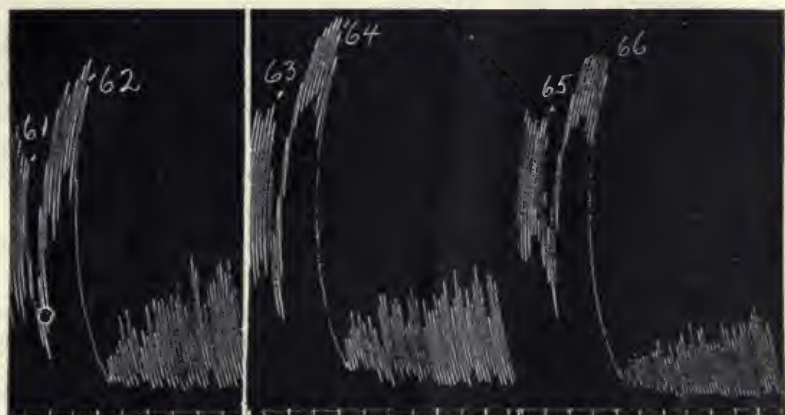


Fig. 8. Intestine tracings. Bloods from cat 406. At 61, 63 and 65 Ringer was replaced by indifferent (venous) blood and this at 62 by indifferent blood to which was added adrenalin to make a concentration of 1:1,330,000; at 64 by the 6th adrenal specimen (collected 5 minutes after intravenous injection of strychnine and 33 minutes after cervical cord section) diluted with 9 volumes of indifferent blood; at 66 by indifferent blood to which was added adrenalin to make a concentration of 1:665,000. All the bloods were diluted with 3 volumes Ringer (the adrenalin bloods after adding the adrenalin). Reduced to one-half.

The 4th specimen, collected after transection, was weaker than 1:4,000,000 (fig. 6, confirmed by another set of observations), stronger than 1:8,000,000, somewhat stronger than 1:6,600,000. It was taken at 1:6,000,000, corresponding to an output of 0.00026 mgm. per minute for the cat, or 0.00012 mgm. per kgm. per minute.

The 6th specimen, collected after injection of strychnine, had so high a concentration of epinephrin that it was diluted with indifferent blood before assay. Diluted with 4 volumes of indifferent blood it was much stronger than 1:2,750,000, i.e., the 6th specimen was much stronger than 1:550,000. It was similarly found to be stronger than 1:265,000 (fig. 7), and slightly weaker than 1:132,000. In another dilution (with 9 volumes of indifferent blood) it was shown to be stronger than 1:275,000, decidedly weaker than 1:66,000, and not very different from 1:132,000 (fig. 8). The segment was more sensitive at this stage and still greater dilution would have been necessary for the best results. The 6th specimen was finally taken at 1:150,000, corresponding to 0.0013 mgm. per minute for the cat, or 0.00062 mgm. per kgm. per minute.

In a previous paper (5) it was shown that the action of strychnine is a lasting one. This has been illustrated in an interesting fashion in the cord transection experiments, for it has been proved that the excitation of the mechanism on which the strychnine increase in epinephrin output depends, once induced, can survive transection of the cervical cord.

Condensed protocol. Cat 418; female; weight, 1.96 kgm.

- 9.10 a.m. Anesthetized with ether, inserted cannulae into trachea, carotid artery and external jugular vein. Obtained a specimen of indifferent (jugular) blood.
- 9.30 a.m. Exposed cord in mid-cervical region. Made cava pocket, tying coeliac axis, renal and superior mesenteric arteries and abdominal aorta (above bifurcation) in addition to the veins entering the cava pocket.
- 10.05 a.m. Cava pocket completed. Collected adrenal blood.
- 10.07½ a.m. First specimen, 1.05 gram in 30 seconds (2.1 grams per minute).
- 10.08 a.m. Second specimen, 3.2 grams in 90 seconds (2.1 grams per minute).
- 10.10 a.m. Blood pressure 105 mm. of mercury.
- 10.14 a.m. Started artificial respiration (cat breathing well spontaneously).
- 10.17 a.m. Intravenous injection of 0.25 mgm. strychnine. Clonic spasms occurred within a few seconds and the blood pressure rose to 120 mm. of mercury.
- 10.21½ a.m. Third adrenal specimen, 0.65 gram in 30 seconds (1.3 gram per minute).
- 10.22 a.m. Fourth adrenal specimen, 3.35 grams in 2 minutes (1.7 gram per minute).
- 10.24 a.m. Blood pressure 94 mm. of mercury.
- 10.30 a.m. Transected cord through origins of 5th pair of cervical nerves (position of cord section verified at autopsy).

10.33 a.m. Blood pressure 24 mm. of mercury. Reflexes exaggerated.

10.34 a.m. Fifth adrenal specimen collected for one minute (not weighed).

10.35 a.m. Sixth adrenal specimen 1.0 gram in 10 minutes (0.1 gram per minute).

Another specimen of indifferent blood was now obtained. Combined weight of adrenals, 0.232, gram.

In figures 9 and 10 are reproduced a small sample of the tracings used in the assay in cat 418.

The 2nd specimen, obtained before injection of strychnine, was found to be stronger than 1:4,500,000 adrenalin, decidedly weaker than 1:3,000,000 and somewhat weaker than 1:3,750,000. It was assayed at 1:4,000,000, corresponding to an output of 0.0005 mgm. per minute for the cat, or 0.00025 mgm. per kgm. per minute.

The 4th specimen, collected after administration of strychnine, but before transection of the cord, was much stronger than 1:3,000,000, somewhat weaker than 1:1,500,000 (fig. 9, confirmed by other observations). It was taken at 1:1,700,000, corresponding to an output of 0.001 mgm. per minute for the cat, or 0.0005 mgm. per kgm. per minute, double the initial output.

The 6th specimen, collected after section of the cord, had a very high concentration of epinephrin. It was diluted with 3 volumes of indifferent blood before assay. Thus diluted it was stronger than 1:750,000 adrenalin, i.e., the 6th specimen was stronger than 1:185,000 (fig. 10, confirmed by other observations). It was probably somewhat weaker than 1:94,000 and was taken at 1:100,000, corresponding to an output of 0.001 mgm. per minute for the cat, or 0.0005 mgm. per kgm. per minute.

Since the output, increased by strychnine, was not changed by the cervical transection it follows that in this animal the influence of strychnine in increasing the epinephrin output was, within the limits of error of the assay, exerted exclusively on the portion of the cord below the transection. It is of interest that in spite of the low blood pressure which followed the transection (to 24 mm. of mercury), spinal shock of the epinephrin secretory mechanism in the thoracic cord, as manifested by a fall in the rate of output, did not occur, the marked diminution in the rate of blood flow through the adrenals being compensated for by the great increase in epinephrin concentration. Where the mechanism has suffered from the condition which we suggest is analogous to spinal shock affecting other centers, a reduced blood flow may be accompanied by a diminished concentration of epinephrin, a combination practically never encountered under the conditions of our

experiments while the central nervous system is intact. The administration of strychnine in this experiment might be said to have constituted a prophylaxis against spinal shock so far as the epinephrin secretory mechanism is concerned.

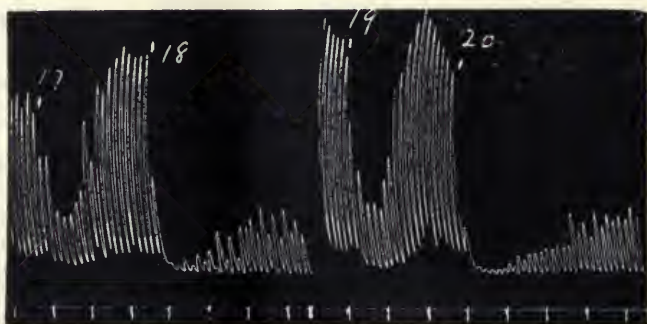


Fig. 9. Intestine tracings. Bloods from cat 418. At 17 and 19 Ringer was replaced by indifferent (venous) blood and this at 18 by the 4th adrenal specimen (collected 5 minutes after intravenous injection of strychnine); at 20 by indifferent blood to which was added adrenalin to make a concentration of 1:1,500,000. All the bloods were diluted with 10 volumes Ringer (the adrenalin blood after adding the adrenalin). Reduced to two-thirds.

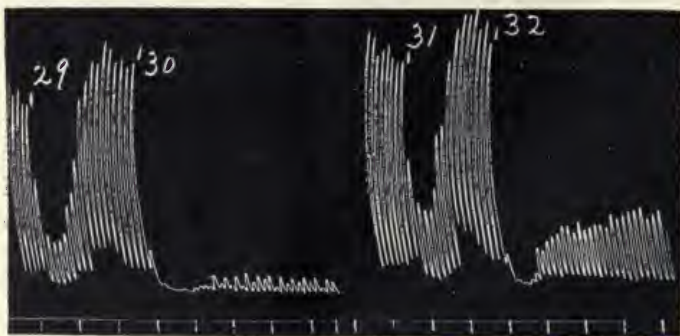


Fig. 10. Intestine tracings. Bloods from cat 418. At 29 and 31 Ringer was replaced by indifferent (venous) blood and this at 30 by the 6th adrenal specimen (collected 18 minutes after intravenous injection of strychnine and 5 minutes after cervical cord section) diluted with 3 volumes of indifferent blood; at 32 by indifferent blood to which was added adrenalin to make a concentration of 1:750,000. All the bloods were diluted with 10 volumes Ringer (the adrenalin blood after adding the adrenalin). Reduced to two-thirds.

In contrast to the action of strychnine on animals with intact central nervous system (5) in which the maximum concentration of epinephrin was not found to surpass substantially the maximum observed without strychnine, the maximum concentration under the influence of strychnine in the cats with cervical cord transection was observed to reach extraordinarily high values (1:70,000 in cat 407; 1:100,000 in cat 418; 1:150,000 in cat 406; 1:250,000 in cat 399). This is consistent with the suggestion expressed in a previous paper (5) that the preliminary diminution in the output produced by strychnine in animals with intact central nervous system may be due to transient stimulation by the drug of a regulatory (inhibitory) mechanism situated higher up and cut off by the cervical transection.

The 2nd specimen from cat 399 (before transection) was shown on intestine segments to be decidedly stronger than 1:7,500,000, stronger than 1:6,000,000, somewhat weaker than 1:4,500,000. It was taken at 1:5,000,000, corresponding to an output of 0.00065 mgm. per minute for the cat, or 0.00025 mgm. per kgm. per minute.

The 4th specimen (after transection) was much stronger than the 2nd. Diluted with 2 volumes of indifferent blood it was still stronger than 1:6,000,000, i.e., the undiluted 4th specimen was stronger than 1:2,000,000. It was found to be decidedly weaker than 1:750,000, weaker than 1:1,000,000 and not far different from 1:1,500,000, corresponding to an output of 0.0002 mgm. per minute, for the cat, or 0.000075 mgm. per kgm. per minute. Considering the small flow this is a fair output, but the striking thing is that the concentration, although increased to more than three times that of the 2nd specimen, corresponding to the diminished blood flow, is not lifted nearly to the possible maximum as would occur with intact cord, whereas when strychnine is given the concentration is increased sixfold, to much beyond the maximum seen without strychnine, while the blood flow is also increasing somewhat.

The assay on intestine segments showed that the 6th specimen (from cat 399) was much stronger than the 4th, much stronger than 1:750,000, much stronger than 1:535,000, much stronger than 1:415,000, stronger than 1:320,000, decidedly weaker than 1:180,000 and not far different from 1:250,000 adrenalin. In making these tests the adrenal blood was diluted with 2 volumes and in other observations with 4 volumes of indifferent blood. It was confirmed on the uterus (fig. 11) that the 6th specimen was much stronger than the others.

That the strychnine action is essentially a central action is best shown by administering the drug to animals from which one adrenal has been previously removed and the nerves of the other cut. Where the output has been reduced below the point of detection or, if detectable, to a small fraction of the normal average, it does not become detectable, or is not increased on injecting strychnine. On the other hand, if a substantial, although of course much reduced output, is present after the adrenal operation, either because the nerves have been incompletely divided or some regeneration has occurred after a long survival period, strychnine causes a definite increase. Illustrations of these facts have already been published in a previous paper (6).

The same thing may be proved by sectioning the adrenal nerves in acute experiments which, however, are on the whole less satisfactory on account of the fall of blood pressure produced by the denervation. A protocol from one of these experiments (cat 431) is given as an example.

*Condensed protocol. Cat 431; female;
weight, 2.05 kgm.*

- 9.20 a.m. Anesthetized with ether; inserted tracheal and jugular cannulae; obtained indifferent (jugular) blood.
- 9.55 a.m. Cava pocket completed. The coeliac axis, renal and mesenteric arteries and abdominal aorta (above bifurcation) were tied in addition to the veins entering the cava pocket. Collected adrenal blood.
- 9.55½ a.m. First specimen, 1.95 gram in 30 seconds (3.9 grams per minute).
- 9.56 a.m. Second specimen, 7.0 grams in 2 minutes (2.5 grams per minute).



Fig. 11. Uterus tracings. Bloods from cat 399. At 62 Ringer was replaced by the 6th adrenal blood specimen (collected 3 minutes after intravenous injection of strychnine and 23 minutes after cervical cord section) diluted with 6 volumes of indifferent blood; at 63 by indifferent (venous) blood; at 64 by indifferent blood to which was added adrenalin to make a concentration of 1:2,250,000; at 65 by the 6th specimen diluted with 4 volumes of indifferent blood. All the bloods were diluted with 3 volumes Ringer (the adrenalin blood after adding the adrenalin). At 66 Ringer was replaced by the 6th specimen; at 68 by the 4th specimen (collected 3½ minutes after cervical cord section); at 69 by the 2nd specimen (collected before cord section). The bloods 66-69 were diluted with 10 volumes Ringer. Reduced to one-half.

- 10.00 a.m. Started artificial respiration (cat still breathing well spontaneously).
10.07 a.m. Intravenous injection of 0.35 mgm. strychnine (sulphate, as in all the experiments).
10.07½ a.m. Clonic convulsions; spontaneous breathing good.
10.22 a.m. Clonic spasms; spontaneous breathing good.
10.23 a.m. Third adrenal specimen, 1.05 gram in 1 minute.
10.24 a.m. Fourth adrenal specimen, 3.85 grams in 3 minutes (1.28 gram per minute).
10.30 to 10.32 a.m. Cut right and left major and minor splanchnics (in abdomen) and extirpated the left semilunar ganglion.
10.34 a.m. Clonic spasms at intervals.
10.38 a.m. Fifth adrenal specimen, collected for 30 seconds.
10.38½ a.m. Sixth adrenal specimen, 2.7 grams in 10 minutes (0.27 gram per minute).

Another specimen of indifferent (venous) blood was now obtained. Combined weight of adrenals, 0.25 gram. Section of nerves verified at autopsy.

Figures 12 to 15 give samples of the tracings used in the assay in cat 431.

The 2nd adrenal specimen was found to be stronger than 1:8,300,000, weaker than 1:5,000,000, weaker than 1:6,600,000 adrenalin. It was assayed at 1:7,500,000, corresponding to an output of 0.00045 mgm. per minute for the cat, or 0.00023 mgm. per kgm. per minute.

The 4th specimen, obtained after administration of strychnine, was decidedly stronger than 1:1,660,000, stronger than 1:830,000. It was much stronger than the 6th specimen, collected after section of nerves to the adrenals in spite of the greatly diminished flow for the 6th specimen. This was evident when both specimens were diluted with 3 volumes of Ringer's solution, but became still more apparent on first diluting each with 3 volumes of indifferent blood and then diluting the mixtures with 3 volumes of Ringer (fig. 12). The 4th specimen diluted with 3 volumes of indifferent blood was weaker than 1:1,660,000 adrenalin, i.e., the 4th specimen was weaker than 1:415,000 (fig. 13). The 4th specimen was finally assayed at 1:700,000, corresponding to an output of 0.0018 mgm. per minute for the cat, or 0.0009 mgm. per kgm. per minute, four times the initial output.

The 6th specimen, diluted with 1 volume of indifferent blood, was weaker than 1:3,300,000, i.e., the 6th specimen was weaker than 1:1,650,000. Undiluted with indifferent blood it was found to be stronger than 1:3,300,000, but decidedly weaker than 1:1,660,000 (fig. 14). It was assayed at 1:2,000,000, corresponding to an output of 0.00013 mgm. per minute for the cat, or 0.00006 mgm. per kgm. per minute, only one-fifteenth of the output under strychnine before section of the nerves.



Fig. 12. Intestine tracings. Bloods from cat 431. At 23 and 25 Ringer was replaced by indifferent (venous) blood and this at 24 by the 4th adrenal specimen (collected 17 minutes after intravenous injection of strychnine) diluted with 3 volumes of indifferent blood; at 26 by the 6th adrenal specimen (collected after section of nerves to both adrenals) diluted with 3 volumes of indifferent blood. All the bloods were diluted with 3 volumes Ringer. Reduced to one-half.

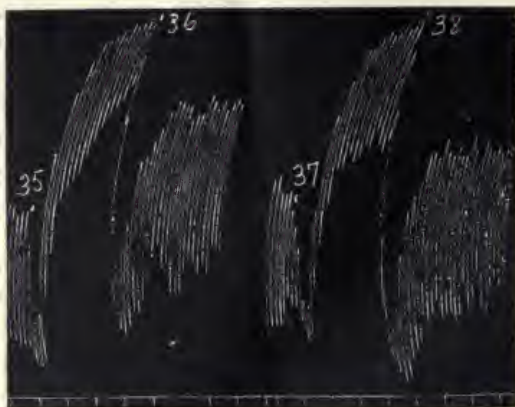


Fig. 13. Intestine tracings. Bloods from cat 431. At 35 and 37 Ringer was replaced by indifferent (venous) blood and this at 36 by the 4th adrenal specimen (collected 17 minutes after intravenous injection of strychnine) diluted with 3 volumes of indifferent blood; at 38 by indifferent blood to which was added adrenalin to make a concentration of 1:1,660,000. All the bloods were diluted with 3 volumes Ringer (the adrenalin blood after adding the adrenalin). Reduced to one-half.

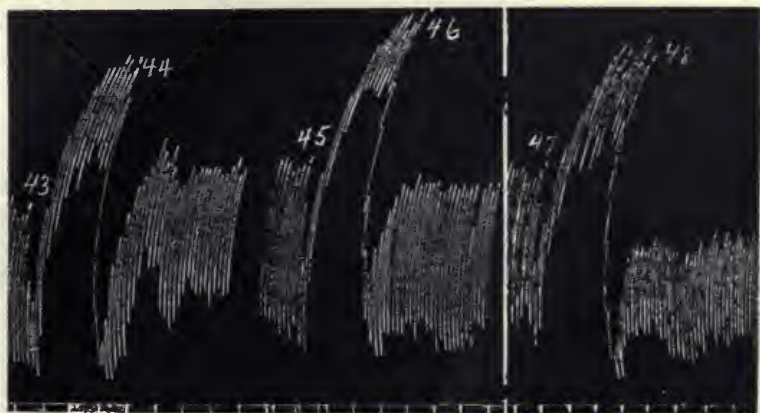


Fig. 14. Intestine tracings. Bloods from cat 431. At 43, 45 and 47 Ringer was replaced by indifferent (venous) blood and this at 44 by indifferent blood to which was added adrenalin to make a concentration of 1:3,300,000; at 46 by the 6th adrenal specimen (collected after section of nerves to both adrenals); at 48 by indifferent blood to which was added adrenalin to make a concentration of 1:1,660,000. All the bloods were diluted with 3 volumes Ringer (the adrenalin bloods after adding the adrenalin). Reduced to one-half.



Fig. 15. Uterus tracings. Bloods from cat 431. At 51 Ringer was replaced by the 4th adrenal specimen (collected after intravenous injection of strychnine) diluted with 3 volumes of indifferent blood; at 52 by the 6th adrenal specimen (collected after section of nerves to both adrenals) diluted with 1 volume of indifferent blood; at 53 by indifferent (venous) blood; at 54 by the 2nd adrenal specimen (collected before injection of strychnine); at 55 by the 4th adrenal specimen; at 56 by the 4th adrenal specimen diluted with 1 volume of indifferent blood. All the bloods were diluted with 3 volumes Ringer. Reduced to two-thirds.

It was confirmed by the uterus that the 4th specimen, diluted with 3 volumes of indifferent blood, was stronger than the 2nd specimen undiluted with indifferent blood, and at any rate not weaker than the 6th specimen diluted with 1 volume of indifferent blood. The 4th specimen was clearly much stronger than the 2nd (fig. 15).

This experiment is a complete contrast to that on cat 318 in which the cervical cord was transected after the administration of strychnine. There the output of epinephrin, increased by the strychnine, went on unchanged. In the present experiment strychnine markedly increased the output, but even incomplete section of the peripheral adrenal secretory paths caused a great diminution.

ACUTE EXPERIMENTS ON OTHER ANIMALS THAN CATS

Table 2 summarizes the results of acute experiments on cervical section in 3 dogs, 2 rhesus monkeys and 1 rabbit. Two experiments on dogs in which the cord was semisectioned in the cervical region are included in the table. In the dogs and monkeys the output after cervical cord transection was always less than before the transection, except in one of the monkeys where the initial output was abnormally low (shock after exposure of the cord). In this case, as noted in cats under similar conditions, transection of the cord caused no further diminution in the epinephrin output. Semisection of the cervical cord in dogs yielded results of the same general character as in cats. In one of the experiments in the table there was no change in the output after the semisection, in the other it was reduced.

In addition to the acute experiments on dogs summarized in table 2, a number of experiments with blood pressure auto-assays were made. For example, in a dog (351) weighing 6.3 kgm. the output was estimated at 0.00015 mgm. per kgm. per minute. The blood pressure was 94 mm. of mercury. The cord was then transected through the 5th cervical pair. The blood pressure fell to 55 mm. of mercury. The output of epinephrin was now estimated at 0.00003 mgm. per kgm. per minute. At the end of the experiment a sample of adrenal blood, collected at the rate of 1.0 gram per minute assayed (on rabbit segments) at 1:3,000,000 corresponding to an output of 0.00005 mgm. per kgm. per minute.

SURVIVAL EXPERIMENTS

In table 3 are displayed the results of experiments on 6 cats and 4 dogs which were allowed to survive transection of the cervical cord for 2 to 13 days. It will be seen that while in most of the animals an output of epinephrin measurable by segments of the sensitiveness employed was present, the output never attained the normal average in animals with intact cord. In the dogs the average output after cervical cord transection is considerably greater than in the cats, the opposite of what is found in the acute experiments (see tables 1 and 2). The suggestion is that the mechanism in the thoracic cord is more easily depressed by the cervical transection (spinal shock), but recovers more easily in survival experiments in the dog than in the cat. We do not know whether this apparently easier recovery means anything more than that the dogs in general in our survival experiments did withstand cervical transection better than the cats. They ate better, passed urine and feces more normally and lived longer. Most of the cats during the time they lived or were permitted to live neither micturated nor defecated, although the bladder was generally much distended with urine. In several instances the bladder was artificially emptied in the cats. This was not necessary in the dogs. It is well known that after these high cord transections one of the most important precautions to be taken is to keep up the body temperature. Although all the animals were kept in a room specially warmed day and night, the dogs were somewhat larger than the cats and therefore it is to be supposed would not cool so easily, but we do not know whether this was a factor in their better condition. Most of the animals were in fair, some (including those taken after the longest periods) in very good condition when sacrificed. It would be desirable to make observations on animals after much longer periods of survival.

While it is clear from these experiments, as from the previous ones, that a substantial liberation of epinephrin, sustained from the thoracic cord, may be present in animals which have survived cervical cord transection 2 to 13 days, it is impossible to say whether with a longer survival period the output would approach more nearly to the normal average or not. Great quantitative and possibly some qualitative changes in metabolism follow such a lesion, and in the present state of our knowledge it would be useless to speculate as to how these might affect the upbuilding or output of epinephrin. Mere inanition for 3 days, and all these animals, of course, take little food for some time

TABLE 2

Acute experiments. Dogs, etc.

NUMBER OF ANIMAL		BEFORE SECTION OF CERVICAL CORD										AFTER SECTION OF CERVICAL CORD										REMARKS
		Body weight	Level of cord section	Combined weight of adrenals	Blood collected	Duration of collection	Blood flow per minute	Blood pressure	Epinephrin concentration	Epinephrin output per minute		Blood collected	Duration of collection	Blood flow per minute	Blood pressure	Epinephrin concentration	Epinephrin output per minute					
				gms.	gms.	sec.	gms.	mm. of mercury		mgm.	Per kilogram	gms.	sec.	gms.	mm. of mercury		mgm.	Per kilogram				
327	7.4	v-vi	0.615	12.35	30	24.7	130	1:15,000,000	0.0016	0.0002		9.5	60	9.5	38	1:40,000,000*		0.00003*				
												10.0	120	5.0	36	1:40,000,000*						
352	6.5	below vi	1.886	9.4	120	4.7	98	1:3,300,000	0.0014	0.0002		6.4	120	3.2	72	1:5,000,000	0.00064	0.0001				
																		After cord section blood pressure fell to 18 mm. of mercury; 300 cc. Ringer injected at once and blood pressure rose to 72 mm. of mercury				
380	5.5	v-vi	1.082	9.7	60	9.7	120	1:4,500,000	0.002	0.00035		2.25	240	(a) 0.56	40	1:1,900,000	0.0003	0.000055				
												4.1	120	(b) 2.05	52	1:8,500,000	0.00025	0.000045				
																		Ringer's solution injected between collection of (a) and (b). 100 cc.				

328	1.87	vii	0.412	4.5	180	1.5	58	1:9,000,000	0.00017	0.0001	4.45	120	2.22	62	1:13,000,000	0.00017	0.0001	50 cc. Ringer injected before and 50 cc. after cord section
250	8.8	vi	0.796	9.2	150	3.7		1:8,000,000	0.00045	0.0004	10.25	120	5.12		1:13,000,000	0.00045	0.0004	Ringer injected after cord section. 100 cc.
276	8.4	below viii	0.947	6.1	60	6.1		1:5,000,000	0.0012	0.00014	5.05	150	2.0		1:9,000,000	0.00022	0.00026	Assay of specimen, taken after cord section, completed next day
379	4.8	above vi	0.894	8.15	90	5.4	140	1:3,000,000	0.0018	0.00037	7.5	180	2.5	97	1:1,400,000	0.0018	0.00037	Both adrenals discharging
382	7.2	iv-v	0.870	10.95	90	(a)7.3	120	1:4,000,000	0.0018	0.00025	5.1	120	(b)2.6	90	1:5,000,000	0.0005	0.00007	(a)before semi-section; (b) after semi-section; (c) after transection below v. Both adrenals discharging
						(c)1.4	44		0.0005	0.0005	4.2	180	(c)1.4	44	1:2,800,000	0.0005	0.00007	

Note. 379 and 382 were semisections (right), the rest transections. 328 was a rabbit, 250 and 276 monkeys, the rest dogs.

* These are the maximum concentrations and output which could have been present. The segment could have detected these concentrations had they been present in the adrenal bloods.

after the operation, does not appear, so far as can be judged from one experiment, to have any notable influence. Thus a cat weighing 2.22 kgm. received no food for 3 days. The stomach and intestines were empty except for some feces in the lower colon. Under urethane specimens of adrenal blood were obtained in the usual way. The 2nd specimen (1.67 gram per minute) was assayed at 1:4,000,000 epinephrin, corresponding to an output of 0.00042 mgm. per minute for the cat, or 0.0002 mgm. per kgm. per minute. The 4th specimen (1.27 gram per minute) was assayed at 1:2,800,000 epinephrin, corresponding to 0.00045 mgm. per minute for the cat, or 0.0002 mgm. per kgm. per minute.

It was shown that strychnine exerted its stimulating effect upon the epinephrin output in these survival experiments, doubtless by acting upon the thoracic cord mechanism, which was already sustaining whatever output was going on. Where the initial output is quite small, as in a cat (424) in which the transection was made not in the cervical region but between the first and second dorsal segments, the relative increase produced by strychnine may be very great.

Condensed protocol. Cat 424; female; weight, 2.1 kgm.

December 9. Excised left superior cervical ganglion.

December 20. Cord transected between origins of 1st and 2nd dorsal nerves. Immediately after cord section (while still anesthetized), the left pupil became wider than the right (both dilated), but on recovery the left pupil contracted and left nictitating membrane came forward as in animals without cord section.

December 24. Condition good. Left pupil contracted and nictitating membrane forward.

10.10 a.m. Administered a little ether to insert tracheal and jugular cannulae and obtained indifferent (jugular) blood. Isolated right and left vago-sympathetic nerves on loose ligatures. Thereafter no more ether was needed.

10.30 a.m. Left pupil contracted and nictitating membrane forward.

Cut left, then right vago-sympathetic nerves. After section of the right vago-sympathetic both pupils became smaller but the left remained smaller than the right; left nictitating forward.

10.45 a.m. Cava pocket completed. The coeliac axis, renal and mesenteric arteries and abdominal aorta (above bifurcation) were tied, in addition to the veins entering the cava pocket. The operative field was of course totally insensitive owing to the cord section.

10.50 a.m. Left pupil wider than right; both nictitating membranes forward.

10.52 a.m. Left pupil wider than right; left nictitating back; left aperture wider than right.

- 10.55 a.m. to 11.20 a.m. Two 2-minute and one 3-minute pocket observations gave no reactions with the pupil; 0.5 cc. 1:650,000 adrenalin gave a good pupil reaction. Collected adrenal blood.
- 11.30 a.m. First specimen, 0.45 gram in 30 seconds (0.9 gram per minute).
- 11.30½ a.m. Second specimen, 5.05 grams in 6 minutes (0.84 gram per minute).
- 11.42 a.m. Started artificial respiration.
- 11.45 a.m. Intravenous injection of 0.5 mgm. strychnine. In a few seconds a tonic convulsion occurred which was followed after a few seconds by clonic spasms. During the spasms the left pupil became maximally dilated, the right pupil about one half dilated, the left nictitating membrane retracted and the right forward. On clipping off the pocket for 3 minutes the left pupil came down somewhat and the nictitating membrane came forward slightly, but the left pupil remained wider than the right.
- 11.51½ a.m. Third adrenal specimen, 0.5 gram in 30 seconds (1.0 gram per minute).
- 11.52 a.m. Fourth adrenal specimen, 3.7 grams in 7 minutes (0.53 gram per minute). On release of the pocket clip after collecting the 4th specimen, a good pupil and nictitating reaction was observed on the left side although the left pupil was already wide). Another specimen of indifferent blood was now obtained.

The 2nd specimen (taken before strychnine injection) was assayed at 1:150,000,000. This corresponds to an output of only 0.0000055 mgm. per minute for the cat, or 0.0000025 mgm. per kgm. per minute, one-hundredth of the normal average. Figure 16 gives some of the tracings used in the assay of the 2nd specimen. It was weaker than 1:130,000,000 adrenalin, stronger than 1:195,000,000. Other tracings, not reproduced, showed that it was much weaker than 1:65,000,000. Fortunately the segment was exceptionally sensitive, as will be seen from the excellent reactions given with blood so poor in epinephrin. It must be remembered that, as applied to the segment, the bloods being diluted with 3 volumes of Ringer's solution, the actual concentrations of epinephrin were only one-fourth of those given. This illustrates a point of technique on which we have often insisted, but which is still overlooked by some investigators, namely, that the mere occurrence of a large inhibitory reaction of an intestine segment gives no information as to the concentration of epinephrin in the blood producing it until the sensitiveness of the segment for epinephrin has been quantitatively determined.

It was shown that the 4th specimen, collected after strychnine, was much stronger than 1:6,500,000, and stronger than 1:1,300,000 adrenalin. Diluted with 3 volumes of indifferent blood it was not much different from 1:3,900,000, i.e., the 4th specimen was not far from

TABLE 3
Cervical cord sections. Survival experiments

NUM- BER OF ANIMAL	BODY WEIGHT	LEVEL OF CORD SECTION	PERIOD OF SURVIVAL	COMBINED WEIGHT OF ADRENALS	BLOOD COL- LECTED	DURATION OF COLLECTION	BLOOD FLOW PER MINUTE	EPINEPHRIN CONCENTRATION	EPINEPHRIN OUTPUT PER MINUTE		REMARKS
									For animal	Per kilo- gram	
<i>Cats</i>			<i>Days</i>	<i>gms.</i>	<i>gms.</i>	<i>sec.</i>	<i>gms.</i>		<i>mgm.</i>	<i>mgm.</i>	
332	2.02	vii	2	0.318	3.95	240	1.0	1:30,000,000*	0.00003*	0.000015*	
336	2.53	vii	3	0.383	4.2	720	0.35	1:3,500,000	0.0001	0.00004	
337	2.6	above vi	3	0.535	3.85	180	1.3	1:30,000,000*	0.00004*	0.000015*	Ether administered between collection of these specimens
					2.75	300	0.55	1:30,000,000*	0.000015*	0.000006*	
346	1.72	vi	7	0.378	4.55	300	0.91	1:25,000,000	0.000036	0.00002	Blood pressure at beginning of experiment was 80 mm. of mercury
					2.35	420	0.34	1:10,000,000	0.000034	0.00002	Ether administered between collection of these specimens
420	1.6	below vi	3	0.290	3.35	600	0.335	1:125,000,000	0.0000025	0.0000015	
422	2.4	below viii	2	0.346	2.55	180	0.85	1:25,000,000	0.000035	0.000015	Pupil assay showed output was not more than 0.000025 mgm. per kgm. per minute
419	1.57	below vii	20	0.461	3.95	240	1.0	1:30,000,000	0.000033	0.00002	

Dogs	4.0	vii	2	0.810	4.2	600	0.42	1:10,000,000	0.00004	0.00001	Very small blood flow. Circulation feeble. Temperature low
330											
345	3.95	vii-viii	8	0.930	8.55 5.0	150 180	3.4 1.7	1:12,000,000 1:8,500,000	0.00028 0.0002	0.00007 0.00005	Ether administered between collection of these specimens
347	2.93	vii-viii	13	1.008	9.7 5.35	120 240	4.8 1.34	1:40,000,000 1:6,600,000	0.00012 0.0002	0.00004 0.00007	0.5 mgm. strychnine administered between collection of these specimens
395	4.5	v-vi	11	0.938	5.8	90	3.9	1:60,000,000	0.000065	0.000015	1st specimen collected without etherization. Blood pressure 94 mm. of mercury
					4.25	90	2.8	1:60,000,000	0.000045	0.00001	2nd specimen collected after etherization. Blood pressure 76 mm. of mercury
386†	5.25	below v	8	0.831	11.25 4.85 4.85	120 120 120	(a)5.6 (b)2.42 (c)2.42	1:8,500,000 1:6,500,000 1:6,500,000	0.00066 0.00035 0.00035	0.00013 0.000065 0.000065	(a) Both adrenals discharging (b) Right adrenal only discharging (c) Left adrenal only discharging

* These are maximum outputs and concentrations which could have been present. The segments, which were not particularly sensitive, could have detected these concentrations had they been present in the adrenal blood.

† 386 is a hemisection experiment (left); the others are transections.

1:1,000,000. It was decidedly stronger than 1:1,625,000 (fig. 17, observations 34 and 36), weaker than 1:650,000 (fig. 17, observations 38 and 40). Another series of observations was made in which the 4th specimen was diluted with 7 volumes and with 14 volumes of indifferent blood, in order to reduce the inhibitory reactions so as to permit of a more exact assay. In this way also it was shown that the specimen was stronger than 1:1,300,000 and weaker than 1:870,000. It was finally taken at 1:1,000,000, corresponding to an output of 0.00053 mgm. per minute for the cat, or 0.00025 mgm. per kgm. per minute, the normal average, and not less than one hundred times the output before strychnine. It was confirmed by the uterus that the

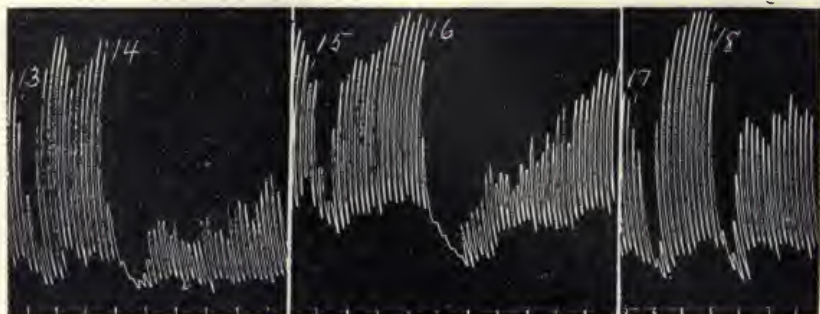


Fig. 16. Intestine tracings. Bloods from cat 424. At 13, 15 and 17 Ringer was replaced by indifferent (venous) blood and this at 14 by indifferent blood to which was added adrenalin to make a concentration of 1:130,000,000; at 16 by the 2nd adrenal specimen (collected before injection of strychnine); at 18 by indifferent blood to which was added adrenalin to make a concentration of 1:195,000,000. All the bloods were diluted with 3 volumes Ringer (the adrenalin bloods after adding the adrenalin). Reduced to one-half.

4th specimen gave a good epinephrin reaction, whereas the 2nd specimen and indifferent blood in the same dilution gave no reaction. The uterus was not particularly sensitive.

Advantage was taken of the fact that it was possible to obtain adrenal vein blood without the administration of an anesthetic to test the question whether ether causes any measurable increase in the output after transection of the cervical cord. In no case was any such effect produced. With intact central nervous system we have previously found that the average output is if anything somewhat less with ether than with urethane (6). An observer who trusts to the (denervated)

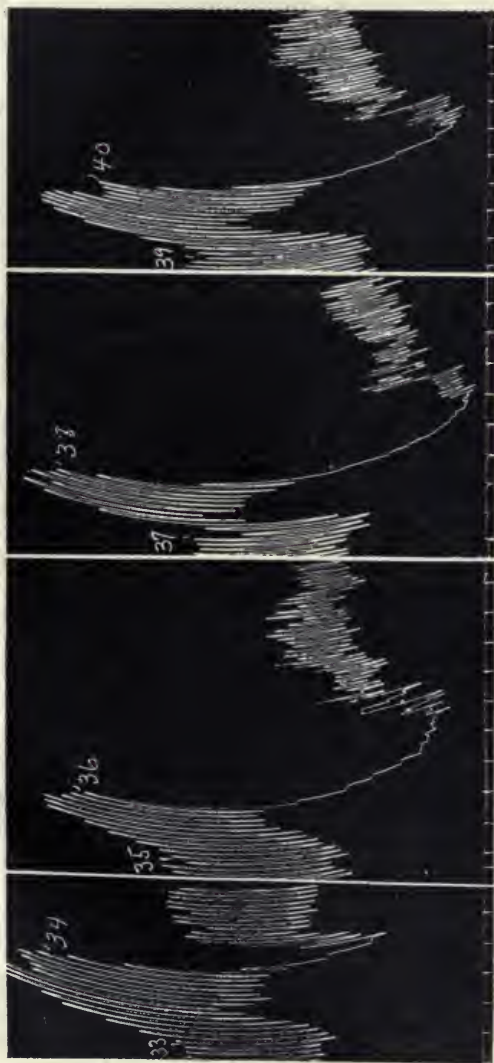


Fig. 17. Intestine tracings. Bloods from cat 424. At 33, 35, 37 and 39 Ringer was replaced by indifferent (arterial) blood and this at 34 by indifferent blood to which was added adrenalin to make a concentration of 1:6,600,000; at 36 by the 4th adrenal specimen (collected 8 minutes after intravenous injection of strychnine) diluted with 3 volumes of indifferent blood; at 38 by indifferent blood to which was added adrenalin to make a concentration of 1:2,700,000; at 40 by the 4th adrenal specimen diluted with 3 volumes of indifferent blood. All the bloods were diluted with 3 volumes Ringer (the adrenalin bloods after adding the adrenalin). Reduced to one-half.

eye reactions alone can easily be deceived by the fact that ether increases the sensitivity of the test object to epinephrin. For instance (in cat 336), a 2-minute pocket without anesthetic gave no reaction. Ether was then administered and now a 2-minute pocket gave an excellent pupil reaction. This was confirmed several times. It was shown that the increased reaction could not be interpreted as due to an augmentation of the epinephrin output by etherization since the reaction to one and the same dose of adrenalin artificially injected was also increased by giving ether. This is illustrated in the following experiment.

Condensed protocol. Cat 423; female; weight, 1.8 kgm.

December 9. Excised left superior cervical ganglion.

December 20. Left pupil contracted and nictitating membrane forward. Transected cord below origin of 8th pair of cervical nerves. Immediately after the cord section the left pupil was wider than the right and the right nictitating protruded more than the left.

December 23. Condition good. Left pupil wider than right as it had been since the cord section, both nictitating membranes slightly forward, apertures of both eyes about the same.

- 9.45 a.m. Administered a little ether to insert tracheal and jugular cannulae. On etherizing, the left pupil became almost maximal, the right pupil dilated but not so widely as the left, the right nictitating protruded more than the left.
- 10.15 a.m. Cava pocket completed. The coeliac axis, renal and mesenteric arteries and abdominal aorta (above bifurcation) were tied in addition to the veins entering the cava pocket.
- 10.16 a.m. Left pupil $\frac{1}{2}$ dilated; right pupil slit-like; left aperture slightly wider than right.
- 10.18 a.m. Pocket observation 2 minutes; no eye reaction.
- 10.22 a.m. Pocket observation 3 minutes; no eye reaction.
- 10.25 to 10.35 a.m. 0.5 cc. adrenalin 1:2,700,000, and 0.5 cc. adrenalin 1:2,000,000 gave no reaction; 0.5 cc. adrenalin 1:1,330,000 gave a pupil reaction in 11 seconds.
- 10.35 a.m. Pocket observation 4 minutes, doubtful reaction if any.
- 10.40 a.m. Etherized to surgical anesthesia.
- 10.45 a.m. Pocket observation 3 minutes gave a pupil reaction in 14 seconds and retraction of nictitating membrane 2 seconds later.
- 10.50 a.m. 0.5 cc. adrenalin 1:2,000,000 gave a very good pupil reaction in 11 seconds.
- 10.55 a.m. 0.5 cc. adrenalin 1:2,700,000 gave a definite reaction in 8.6 seconds; 0.5 cc. adrenalin 1:4,000,000, doubtful if any reaction.

This experiment brings out another point, namely, the reversal of the eye phenomena after section of the cord, which Schafer (7) has called attention to as occurring after section of the cervical sympathetic

on the side opposite to that on which the superior cervical ganglion has been excised. The same reversal was observed on section of the cord just below the 8th cervical segment in cat 422. The left superior cervical ganglion (in cat 422) was excised a fortnight before section of the cord. Up to the time when the cord was divided the left pupil was smaller than the right and the left nictitating membrane was less retracted than the right. On section of the cord the left pupil became wider than the right and the right nictitating protruded further than the left. This condition persisted for the two days during which the animal was allowed to survive. At the end of the experiment in which adrenal blood was obtained, the observation was made when the animal was being bled to death that with the onset of asphyxia the left pupil dilated widely but not so widely as the right which went to maximal dilatation. The right nictitating became completely retracted, while the left was still visible. The right aperture became wider than the left and remained so until death. No anesthetic was administered as of course the whole operative field was completely insensitive. In cat 424, in which the cord was transected between the first and second dorsal segments, the reversal of the eye phenomena was not seen.

OBSERVATIONS ON THE ADRENAL EPINEPHRIN STORE AFTER TRANSECTIONS OF THE CORD

In many of the animals the epinephrin store of the adrenals was estimated at the end of the experiment. The results are collected in table 4. In the two acute experiments, one on a monkey, the other on a dog, the load is less than would be expected in a normal animal killed without anesthetic. But these animals were necessarily anesthetized for some time before the cord transection, and thereafter, nothing can be deduced from this as to any influence of the cord lesion on the store. In the survival experiments, in many of which little or no anesthetic was required for the final experiment, the field of operation being insensitive, there is no conspicuous difference in the store from what would be expected in normal animals. If it looks somewhat too low in some of the animals, it is fully as great as the normal load in others. The effect of post-operative depletion, following the initial operation in which the cord was divided cannot be altogether excluded, although it is not evident. For example, in cat 419, which was taken 20 hours after the cord section, there was a full load.

TABLE 4

NUM- BER OF ANIMAL	KIND OF ANIMAL	WEIGHT OF ADRENALS		EPINEPHRIN CONTENT		REMARKS
		Left	Right	Left	Right	
		grams	grams	mgm.	mgm.	
333	Cat	0.231	0.230	0.16	0.16	Cervical cord section below 6th seg- ment: survival 3 days
335	Cat	0.151	0.155	0.16	0.16	Cervical cord section above 7th seg- ment: survival 3 days
336	Cat	0.197	0.186	0.14	0.14	Cervical cord section through 7th seg- ment: survival 3 days
337	Cat	0.257	0.278	0.16	0.17	Cervical cord section above 6th seg- ment: survival 3 days
346	Cat	0.192	0.186	0.23	0.23	Cervical cord section through 6th segment: survival 7 days
348	Cat	0.228	0.224	0.26	0.26	Dorsal cord section below 5th seg- ment: survival 6 days
419	Cat	0.241	0.220	0.26	0.26	Cervical cord section below 7th seg- ment: survival 20 hours
420	Cat	0.152	0.148	0.15	0.15	Cervical cord section below 6th seg- ment: survival 3 days
421	Cat	0.230	0.218	0.22	0.22	Cervical cord section through 7th segment: survival 4 days
422	Cat	0.184	0.162	0.13	0.14	Cervical cord section below 8th seg- ment: survival 2 days
423	Cat	0.208	0.200	0.19	0.19	Cervical cord section below 8th seg- ment: survival 3 days
338	Dog	0.397	0.372	0.42	0.40	Cervical cord section below 7th seg- ment; survival 5 days
345	Dog	0.480	0.450	0.46	0.47	Cervical cord section below 7th seg- ment: survival 8 days
347	Dog	0.488	0.520	0.64	0.66	Cervical cord section below 7th seg- ment: survival 13 days
349	Dog	0.722	0.708	1.31	1.33	Dorsal cord section below 5th seg- ment: survival 7 days
352	Dog	0.964	0.922	0.41	0.42	Acute experiment: morphine and ether: cervical cord section below 6th segment: also dorsal section
394	Dog	0.406	0.352	0.47	0.46	Cervical cord section below 5th seg- ment: survival 8 days
395	Dog	0.520	0.418	0.48	0.48	Cervical cord section below 5th seg- ment: survival 11 days
276	Monkey	0.480	0.467	0.20	0.20	Urethane anesthesia: acute experi- ment: cord section below last cer- vical segment: adrenals assayed next day

TABLE 5

NUMBER OF ANIMAL	WEIGHT OF ADRENALS		EPINEPHRIN CONTENT		REMARKS
	Left	Right	Left	Right	
	grams	grams	mgm.	mgm.	
339	0.212	0.186	0.26	0.20	Under ether cervical cord sectioned below 7th segment, left adrenal excised and ether administered for 3 hours and 15 minutes, then removed right adrenal
340	0.160	0.148	0.16	0.16	Cat died 8 days after denervation of left adrenal and 2 days after cervical cord section between 5th and 6th segments
341	0.330	0.300	0.34	0.34	Left adrenal denervated 9 days; cervical cord transected below 6th segment 3 days; cat dying when adrenals were removed
342	0.344	0.320	0.31	0.31	Left adrenal denervated 9 days; cervical cord transected below 6th segment 3 days; ether 2 hours; cat in moribund condition
343	0.210	0.236	0.25	0.16	Under ether sectioned cervical cord (between 5th and 6th segments); excised left adrenal; then morphine 60 mgm., injected hypodermically; removed right adrenal 5½ hours later
344	0.22	0.24	0.21	0.21	Under ether, sectioned cervical cord (above 6th segment); excised left adrenal; then 6 cc. of 2 per cent solution of β -tetrahydronaphthylamine injected hypodermically; right adrenal excised 5 hours later

In table 5 are given the results on 6 cats in which a differential effect on the two adrenals could have been manifested, either because one gland had been previously denervated or because one adrenal was removed early and the other at the end of the experiment. In the one experiment tried, morphine seemed to produce its ordinary differential depletion after cervical transection, qualitatively at any rate. It would be impossible without more experiments to know whether the effect was as great quantitatively as with intact cord. In the one experiment made, β -tetrahydronaphthylamine caused no differential effect. Both with morphine and β -tetra the usual symptoms were elicited, so far as they could be manifested by the portion of the animal innervated from above the cord lesion. In 3 cats, dead or moribund at the time the adrenals were removed, the store was the same in each gland. This has little significance, as the same may be seen with intact

cord, although often in animals dying in the laboratory after section of the nerves of one adrenal a marked difference in the store has been found.

SUMMARY

1. Our previous work on the liberation of epinephrin after the transection of the cervical cord at various levels in acute experiments on cats has been confirmed and extended. The output may be unaltered by the transection, or it may be diminished. Evidence is given that when the output is diminished this is due to "spinal shock" of the mechanism in the thoracic cord concerned in sustaining the epinephrin output. When the bulb and brain were eliminated by a bloodless method (ligation of the head arteries) the output remained uniformly undiminished.

2. In dogs the epinephrin output in acute experiments was always diminished by transection of the cervical cord, owing, it is suggested, to the greater susceptibility to spinal shock of the epinephrin secretory mechanism. The two monkeys examined showed in this regard the same behavior as the dogs.

3. In survival experiments, the output never equalled the average ordinary output, although it was often substantial. In these experiments it appeared that in dogs the output approached more nearly to that found in animals with intact nervous system than in cats, the opposite of what was seen in the acute experiments, as if the secretory mechanism in dogs, although more easily depressed by the spinal section, recovered to a greater degree in the relatively short periods for which the animals were kept alive (up to 13 days). It is not known whether the better general condition of the dogs than of the cats after the operation is a factor in this recovery.

4. Strychnine increases markedly the epinephrin output after transection of the cervical cord both in acute and survival experiments. The action is central (on the thoracic cord).

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DEMONSTRATION THAT THE SPONTANEOUSLY LIBERATED EPINEPHRIN CAN EXERT AN ACTION UPON THE HEART

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In the course of our experiments upon the epinephrin output, evidence has accumulated that the steady spontaneous discharge is of sufficient magnitude to cause definite physiological effects in the organism. For example, in the latter part of the experiment on cat 313 in the paper on strophanthin a series of observations were made which constitute a demonstration that the epinephrin discharged from the adrenals at the ordinary rate can exert a clearly detectable action upon the heart. We have other experiments in which strophanthin was not given which prove the same thing and one of them will also be referred to, as it seems to us more important to establish that the amount of epinephrin spontaneously given off can and does produce definite physiological reactions, than to deduce from the effects of the monstrous doses of adrenalin which have been employed in investigating its so-called "physiological action" what epinephrin must do when liberated naturally into the blood stream.

In the experiment on cat 313 about eight minutes after the second dose of strophanthin an irregularity developed in the blood pressure tracing, which was not connected with any change in the respiration. The animal was breathing naturally and artificial respiration had not been employed. At this time an injection of 0.5 cc. of 1:150,000 adrenalin was made into the jugular vein (fig. 1, observation 34). The irregularity, which was unquestionably due to a cardiac arrhythmia, became more pronounced. At 35, one minute after the adrenalin injection,

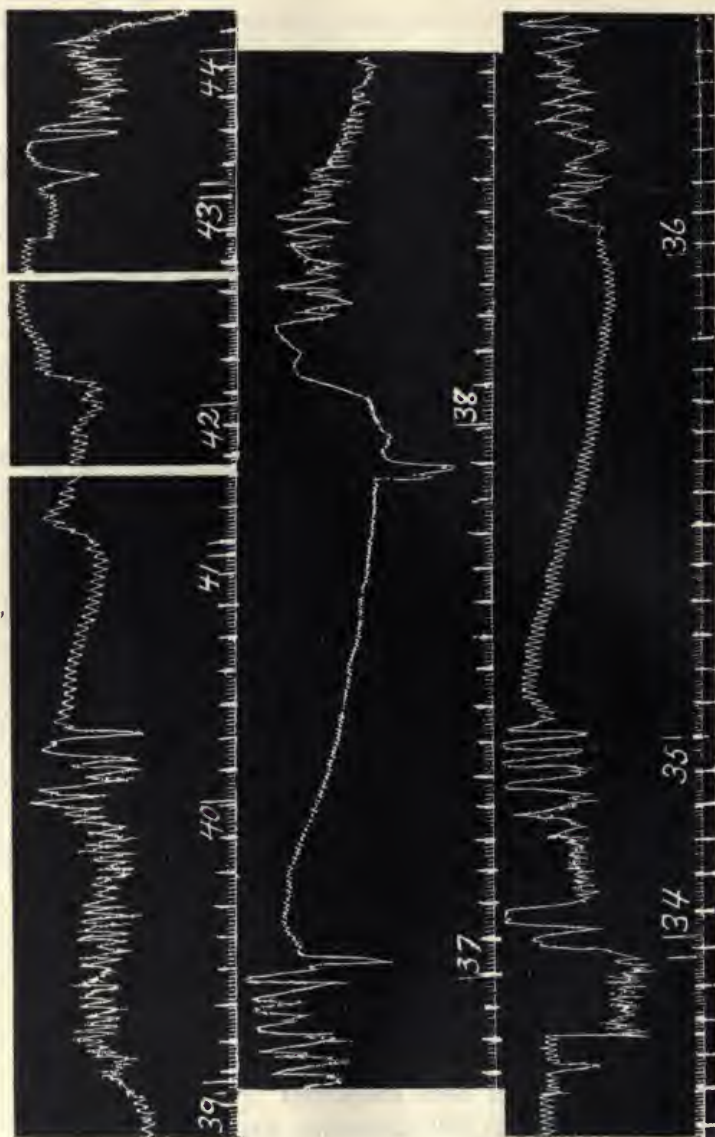


FIG. 1. BLOOD PRESSURE TRACING FROM CAT 313, AFTER ADMINISTRATION OF STROPHANTHIN

Showing the effect of exclusion of the naturally liberated epinephrin upon the cardiac irregularity, and demonstrating that the epinephrin discharged from the adrenals exerts an action upon the heart. For details, see text. The zero line, coincident with the time trace, has been moved up 30 mm. for observations 34 to 36; 23 mm. for observations 37 and 38; and 28 mm. for observations 39 to 44. Time, seconds and 8 seconds.

the cava pocket was closed off and the irregularity completely disappeared after a latent interval, allowing for lost time in making the signal mark, of six or seven seconds. During the two minutes for which the pocket was closed, no trace of the irregularity could be discerned. The exclusion of the epinephrin which had previously been entering the blood at the rate of about 0.001 mgm. per minute, must accordingly have been responsible for the marked change in the heart's action, and, therefore, this epinephrin discharge must have previously been exerting an effect upon the heart which contributed to the arrhythmia. The action of the previously injected adrenalin, if this had anything to do with the intensification of the arrhythmia, as it doubtless had from other observations made later, would almost certainly have passed off before the pocket was closed, as shown by the duration of the effect of adrenalin in other observations on this cat. But it is a matter of indifference as regards the cogency of the demonstration whether this was the case or not, since the experiment was repeated again and again without any further injection of adrenalin and with the same result. At 36 (fig. 1) the pocket was opened, the accumulated epinephrin entered the circulation and after a latent period of seven or eight seconds, the irregularity reappeared. The entrance of the accumulated epinephrin was, therefore, responsible for the reestablishment of the cardiac irregularity. This would not prove anything as to the action of the naturally liberated epinephrin *discharged at its normal rate* on the heart, since it had been accumulated for two minutes and was then allowed to enter the blood stream rather abruptly. But now at 37, when this accumulated epinephrin must have long since disappeared, the pocket was again closed, and again after about the same latent period the heart began to beat regularly, showing that the arrhythmia was conditioned by the epinephrin discharged at the ordinary rate from the adrenals. The pocket was kept closed for two minutes. Towards the end of this period in spite of the exclusion of the epinephrin, a brief recurrence of the irregularity took place, but this passed off and did not recur till after the opening of the pocket at 38, after a latent period

of about twenty seconds. At this stage the tendency to arrhythmia was less pronounced than earlier and in about a minute after the opening of the pocket the heart was beating with fair, although not perfect regularity. At this point, one and a half minutes after the opening of the pocket, 0.5 cc. of a 1:300,000 solution of adrenalin was injected into the jugular (observation 39), markedly intensifying the irregularity after a brief latent period. A minute later the pocket was closed at 40 and after an interval of somewhat more than fifteen seconds the curve again became regular. At 41, 0.5 cc. of 1:300,000 adrenalin was again injected, while the pocket was still closed, but now the heart was in such a condition that no noticeable irregularity was produced. The pocket was opened at 42, after having been closed for two minutes. The naturally secreted epinephrin accumulated during this time, although it caused a fair rise of blood pressure, produced, like the artificially introduced adrenalin, no irregularity at this stage. Two minutes later (at 43) a third dose of strophanthin (0.05 mgm.) was injected, developing at once a marked cardiac irregularity. At 44 the cava pocket was closed off, but the blood pressure fell rapidly and the animal died.

The conclusion which we wish to draw from this experiment and which we think follows inevitably, is that the naturally liberated epinephrin entering the circulation at its normal rate was producing a demonstrable effect upon the heart. The precise nature of the action and the precise point of attack of the epinephrin need not be discussed at present. But it seems fairly obvious that the strophanthin arrhythmia was heightened, or the threshold of the strophanthin, action lowered by the naturally discharged epinephrin. At a stage when the strophanthin action had worn off and adrenalin, in such doses as are employed for assaying the amount of the natural output, no longer developed the irregularity, the naturally secreted epinephrin was equally without effect.

It does not necessarily follow from these observations that the susceptibility of the heart is increased by strophanthin for all the reactions which epinephrin in the amounts in which it is normally liberated can exert upon the heart. But if this were the

case for the accelerating affect, for instance, it is easy to see that the consideration which led Richards and Wood to suspect that strophanthin might increase the epinephrin output would bear no such significance. They state that they were led to this idea by observing that the accelerating effect of strophanthin upon the heart was much more common with the heart in situ than with the isolated organ. There are, of course, many possible causes for such a difference between an isolated organ and the same organ in situ. But if the epinephrin discharged from the adrenals has anything to do with it, it might be explained equally well on the hypothesis that the naturally secreted epinephrin produced the effect, not because it was liberated at an augmented rate but because the test object had been rendered more sensitive to it. That the adrenal vein blood when collected in a cava pocket and then released, causes a marked acceleration of the heart in animals to which no drug except an ordinary anesthetic has been administered, is easy to demonstrate. To show an effect of the steady normal discharge of the epinephrin on the heart rate is another matter. As we have already pointed out in the case of the blood pressure a negative result of exclusion of the adrenal blood when the regulative nervous mechanism is still intact has little value.

It is not only in animals under the influence of strophanthin that the normally secreted epinephrin has been shown to have an influence on the heart. In figure 2 are reproduced portions of a blood pressure tracing from a cat (265) anesthetized with urethane, which exhibits phenomena fully as striking and incapable of any other explanation than that the epinephrin discharged steadily into the blood stream by the adrenals at a rate well within the normal range was exerting a definite effect upon the heart. The animal, a male, weighing 3.78 kgm., received 6 grams urethane by stomach tube and was deeply anesthetized in half an hour. The vagi were cut at the beginning of the experiment. It was noted at the beginning on palpating the pulse that the heart was irregular and that every few beats a pulsation seemed to be dropped. It was estimated by the method of auto-assay by blood pressure reactions, that the epinephrin

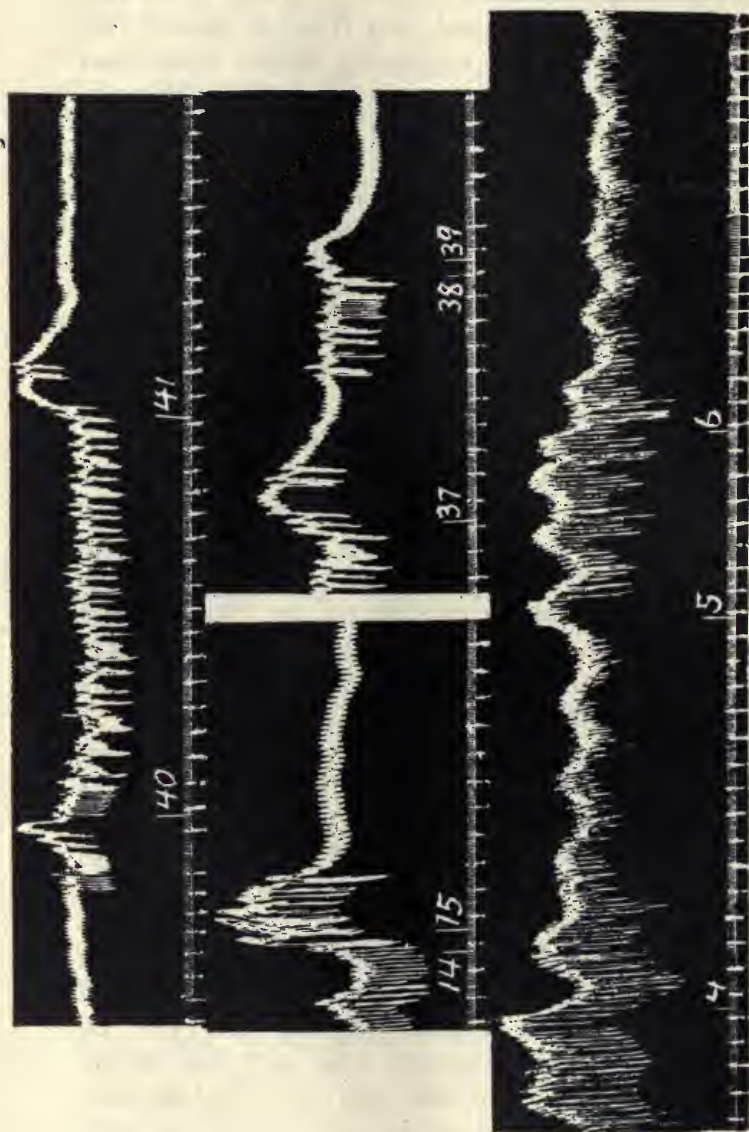


FIG. 2. BLOOD PRESSURE TRACING FROM CAT 265, ANESTHETIZED WITH URETHANE

Showing the effect of exclusion of the naturally liberated epinephrin on a cardiac arrhythmia present at the beginning of the experiment, and demonstrating that the epinephrin discharged from the adrenals exerts an action upon the heart. For details, see text. The zero line, coincident with the time trace, has been moved up 42 mm. for observations 4 to 6; 40 mm. for observations 14 and 15; and 20 mm. for observations 37 to 41. In this figure, as in figure 1, the finer details of the irregularity in the curve have been somewhat obscured in the reproduction.

output was not more than 0.0002 mgm., or less than 0.00015 mgm. per kilogram per minute, i.e., rather under than above the normal average. A more exact assay could not be made on account of the irregularity of the heart. The adrenals weighed 0.755 gram. It was observed that on closing off the cava pocket, the appearance of the blood pressure curve altered in such a way as to indicate a marked diminution in the cardiac irregularity, as shown for instance in figure 2 at 4 where the pocket was closed. The change could have nothing to do with any effect which the abstraction of such a small quantity of blood as was collected in the pocket (about 1 to at most 2 cc.) could have had on the filling of the heart. The latent period of the effect (eight or ten seconds) was such as would be expected if it was due to the disappearance of an action on the heart which was being exerted by the small steady output of epinephrin into the blood stream before the closing of the pocket. A similar pocket observation earlier in the experiment gave the same result. At 5 the pocket was released, the pent up adrenal blood passed into the circulation and after the usual latent period of eight or ten seconds, the irregularity of the heart beat returned. At 6 the pocket was again closed and the curve altered precisely as before. The same was the case with another pocket observation a few minutes later. The opening of the pocket augmented the irregularity and so did the injection of adrenalin at this stage, in such amounts as were used to assay the epinephrin output. A very large amount of epinephrin, on the other hand (0.5 cc. of a 1: 35,000 solution) caused the irregularity to disappear, perhaps after a brief increase (fig. 2, injection from 14 to 15). Precisely the same reversal of the original effect was seen at a later stage in the experiment when the cava pocket was closed off, the irregularity now developing or becoming greater during the period of closure of the pocket and disappearing, after the usual latent period, when the pocket was opened and the accumulated epinephrin reached the heart. At 37, for example, a pocket which had been closed three minutes, was opened. When the pocket was closed the heart, after the injection of 0.5 cc. of 1: 70,000 adrenalin, a strong dose, was beating regularly. Irregu-

larity developed during the time the pocket was closed and is obvious on the blood pressure curve before 37. After the opening of the pocket the irregularity disappeared for a time, of course after a certain period of delay. The amount of epinephrin collected in the pocket would not be half as much as the dose of adrenalin injected before the closure of the pocket and it did not remove the irregularity for as long a time. At 38 to 39, 1 mgm. of strychnine sulphate was injected intravenously. The irregularity of the heart rapidly disappeared, the period of delay being less than before. The strychnine began to enter the circulation a little before the first signal mark. The second mark indicates the end of the washing in of the dose of strychnine with the usual small quantity of salt solution. There was at this time no noticeable effect on the reflex excitability, but it must be remembered that the cat was deeply anesthetized with urethane. We have shown that strychnine can markedly augment the epinephrin output, and the most natural explanation of the effect upon the heart is that it was due to epinephrin. If the output was augmented, the latent period of the effect would be diminished. A little before 40 the irregularity returned and was decidedly increased by excluding the epinephrin when the pocket was closed at 40. After three minutes, the pocket was opened at 41 and with the arrival of the epinephrin at the heart the irregularity disappeared completely for two to three minutes. When it returned it was much less prominent than before, but was still increased somewhat by closing off the cava pocket and abolished by opening it. This was the case even when the irregularity had been reduced to the occurrence of a group of two or three larger strokes on the blood pressure curve at long intervals. Fourteen minutes after the first dose of strychnine when practically all irregularity had disappeared, another dose of 1 mgm. was given. The reflex excitability was now distinctly increased. Exclusion of the epinephrin caused no return of irregularity until the pocket had been closed for more than two minutes, when three or four small groups of irregular beats were seen at intervals of twelve or fifteen seconds. With the release of the pocket, these groups completely disappeared.

As in the experiment with the strophanthin irregularity we prefer to draw only the conclusion, which seems incontrovertible, that the spontaneously discharged epinephrin was in this animal exerting a demonstrable influence upon the heart. There is nothing really puzzling in the fact that at one stage the exclusion of the epinephrin should diminish or abolish the irregularity, and that the release of the adrenal blood should increase or develop it, while at a later stage in the experiment precisely the opposite effect should be produced. For in a given state of the heart, the amount of epinephrin reaching it from the adrenals may be only sufficient to encourage the development of extra contractions instead of causing an equable acceleration of regular beats. In other words, while inciting the heart to hasten, it may cause it to stumble, whereas with a larger dose of epinephrin or an altered susceptibility of the heart to the same dose the improvement of the working power of the heart by the epinephrin may enable the stumbling heart to rid itself of the arrhythmia while accelerating its beat. The difference would then be something like the difference between the effect of flogging an exhausted and a fresh horse. For our purpose the fact that the response of the heart (as regards the arrhythmia studied) to the exclusion of the naturally liberated epinephrin, was different in the two stages of the experiment, only renders the demonstration of the action of the epinephrin more conclusive. For with the reversal of the response of the heart to artificially injected adrenalin came the reversal of its response to the natural epinephrin discharge. When the adrenal blood is excluded from the circulation other substances than epinephrin, if other substances are liberated from the adrenals into the blood stream, will of course, be excluded also. Such bodies might also affect the heart. But from the exact reproduction by artificially introduced adrenalin of the effects of the adrenal blood upon these cardiac irregularities it is clear that in the phenomena studied it is the epinephrin which is the effective factor.

We have already pointed out that investigations which elucidate the factors concerned in sustaining and modifying the epinephrin output may easily come to have a bearing upon the

output of other adrenal products, e.g., the substance whatever it may be in virtue of which the cortex exerts that action upon the organism which is indispensable for life. On the other hand, it is fairly clear that if the nervous system exerts an influence upon the output of such substances this influence is less complete than in the case of epinephrin.

SUMMARY

It was demonstrated that the epinephrin passing into the blood stream from the adrenals at the ordinary rate can exert a definite action upon the heart. This was clearly shown by the marked effect produced upon the cardiac irregularity evoked by strophanthin, when the adrenal blood was temporarily excluded from the circulation or allowed to enter it. Similar observations were made in a case of cardiac irregularity occurring in the absence of strophanthin. It was proved by the artificial administration of adrenalin that the constituent in the adrenal blood responsible for the observed effects was epinephrin.

FURTHER OBSERVATIONS SHOWING THAT EPINEPHRIN FROM THE ADRENALS IS NOT INDISPENSABLE

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We showed in a previous paper (1) that cats live indefinitely in good health after excision of one adrenal and section of the nerves of the other, an operation which either abolishes the output of epinephrin from that adrenal or reduces it to a small fraction of the normal. It is well known that a certain proportion of rabbits survive double adrenalectomy, in our experience something like 20 per cent (2). Whether this is due in all cases to the presence of accessory adrenals or not, it is clear that in rabbits also the epinephrin secretion of the adrenals is not indispensable since accessory adrenals consist only of cortical tissue. In this investigation we have extended the observations to dogs and monkeys, with the same result; neither the length of life nor the health of the animals could be shown to be in any way affected by removal of one adrenal and section of the nerves of the other. Some protocols with samples of tracings used in assaying the epinephrin in the adrenal vein blood at the time the animals were sacrificed are reproduced.

CONDENSED PROTOCOL

Dog 241. Male. Weight 6.92 kgm.

October 21, 1918. Right adrenal excised. It weighed 0.55 gram and contained 0.47 mgm. epinephrin. Nerves of left adrenal cut (major and minor splanchnics, and all twigs seen going to the adrenal).

November 11, 1918. Condition excellent, weight 7.5 kgm.

Under morphine and ether, obtained a specimen of blood from the jugular vein. Then made cava pocket in usual way, tying abdominal aorta but not the coeliac and mesenteric arteries.

Collected the following specimens of adrenal blood:

1st specimen, 2.1 grams in $1\frac{1}{2}$ minutes (1.4 grams per minute)

2nd specimen, 6.85 grams in 5 minutes (1.37 grams per minute)

3rd specimen, 6.0 grams in 5 minutes (1.2 grams per minute)

4th specimen, 3.3 grams in 4 minutes (0.8 gram per minute)

Now obtained additional indifferent blood from the jugular and a sample from the abdominal aorta. The left adrenal weighed 0.562 gram and contained 0.66 mgm. epinephrin.

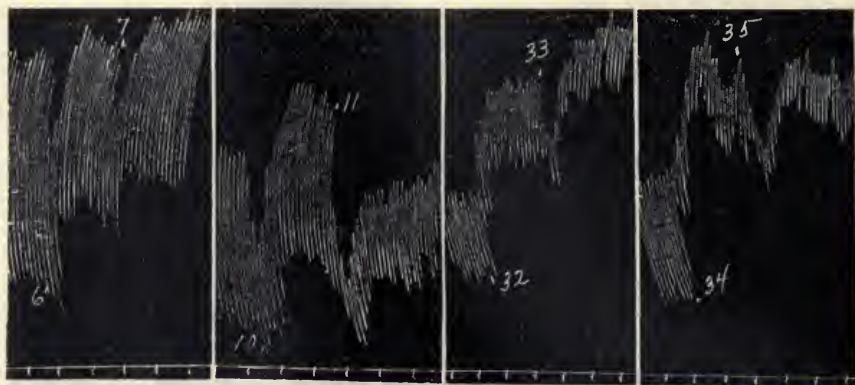


Fig. 1. Intestine tracings. Bloods from dog 241. At 6, Ringer was replaced by jugular blood and this at 7 by the 4th adrenal specimen; at 10 Ringer was replaced by jugular blood, and this at 11 by jugular blood to which was added adrenalin to make a concentration of 1:62,500,000. The bloods were diluted with three volumes Ringer (the adrenalin blood after adding the adrenalin); at 32 Ringer was replaced by jugular blood and this at 33 by the 3rd adrenal specimen; at 34 Ringer was replaced by jugular blood and this at 35 by jugular blood to which was added adrenalin to make a concentration of 1:95,000,000. The bloods were diluted with one volume Ringer (the adrenalin blood after adding the adrenalin). As in all the other figures, time is marked in half-minutes. (Reduced to one-half.)

The epinephrin assay of the adrenal blood (samples of the tracings are given in fig. 1) showed that the 4th specimen was much weaker than 1:62,500,000 adrenalin¹ (observations 7 and 11). Other tracings,

¹ All our results on the concentration and output of epinephrin are given in terms of the base, the amount of the base in Parke, Davis & Co.'s adrenalin hydrochloride solution being assayed by the colorimetric method of Folin, Cannon and Denis. The assay of freshly opened bottles has usually yielded from 65 to 80 per cent adrenalin base. A bottle which is used more than once is always re-assayed from time to time.

not reproduced, indicated that it did not differ much from 1: 125,000,-000. The 2nd and 3rd specimens, corresponding to the greater flow, were weaker than the 4th, the 2nd being decidedly weaker than 1: 125,-000,000 adrenalin. This was confirmed by several observations (not reproduced). In figure 1 it is shown that the 3rd specimen was distinctly weaker than 1: 95,000,000 adrenalin (observations 33 and 35).

TABLE 1

NUMBER OF DOG	BODY WEIGHT	WEIGHT OF ADRENALS	BLOOD FLOW				EPINEPHRIN CONCENTRATION	EPINEPHRIN	
			Grams	Minutes	Seconds	Grams per minute		Per animal per minute	Per kilogram per minute
	<i>kgm.</i>	<i>grams</i>						<i>mgm.</i>	<i>mgm.</i>
15	8.5		24	3		8.0	1: 3,500,000	0.0024	0.0002
			22	3		7.3	1: 3,000,000	0.0024	0.0002
17	6.7	0.65	20	4	45	4.2	1: 3,300,000	0.0013	0.0002
18	7.7	0.78	13	2		6.5	1: 6,000,000	0.001	0.00015
206	6.2	0.905	22.9	2		11.5	1: 7,500,000	0.0015	0.00025
207	7.3	1.2	16.1	2		8.0	1: 8,000,000	0.001	0.00014
221	11.3	1.8	17.4		30	34.8	1: 18,000,000	0.002	0.00018
245	9.5	1.55	10.0	1		10.0	1: 3,750,000	0.0027	0.00028
246	7.5	1.26	7.7		30	15.4	1: 13,000,000	0.0012	0.00016
247	7.25	1.12	4.8	1	30	3.2	1: 1,800,000	0.0018	0.00025
248	4.6	0.8	9.7	1		9.7	1: 13,000,000	0.0008	0.00017
249	6.6	0.848	9.6	1		9.6	1: 6,250,000	0.0015	0.00023
256	6.2	0.733	10.6	1	30	7.1	1: 6,500,000	0.0011	0.00018
257	6.0	0.908	8.65	1		8.65	1: 6,650,000	0.0013	0.0002
263	4.4	0.78	6.15	1		6.15	1: 3,800,000	0.0016	0.00036
280	7.0	1.8	12.5	1		12.5	1: 10,000,000	0.0013	0.00018
306	5.05	0.705	8.2	1		8.2	1: 8,000,000	0.001	0.0002
307	4.6	0.831	11.05	1	30	7.4	1: 4,800,000	0.0015	0.00033

The first three dogs in the table were anesthetized with ether alone, the rest with morphine and ether.

Taking the 4th adrenal specimen at 1: 120,000,000 we get an output of 0.0000065 mgm. per minute for the dog, or 0.0000009 mgm. per kilogram of body weight per minute. The normal average output for dogs under the conditions of our experiments, as assayed on rabbit intestine (and uterus) segments, is 0.00022 mgm. per kilogram per minute (table 1). The output had, therefore been reduced by the operation in this animal to less than $\frac{1}{250}$ of the normal.

CONDENSED PROTOCOL

Dog 242. Female. Weight 5.21 kgm.

October 28, 1918. Right adrenal excised. It weighed 0.425 gram and contained 0.47 mgm. epinephrin. Nerves of left adrenal cut (major and minor splanchnics and all branches seen entering gland; superior mesenteric ganglion and part of coeliac ganglion also excised).

November 22, 1918. Condition excellent. Weight 5.6 kgm. Under morphine and ether collected jugular blood, then made cava pocket in usual way, tying abdominal aorta but not the coeliac and mesenteric arteries.

Collected adrenal blood:

11.35 a.m. 1st specimen 0.6 gram in 30 seconds (1.2 grams per minute). 2nd specimen 6.15 grams in 5 minutes (1.2 grams per minute)

11.55 a.m. Injected intravenously 0.3 mgm. strychnine sulphate—clonic spasms

11.56 a.m. 3rd specimen, 0.75 gram in 30 seconds (1.5 grams per minute). 4th specimen, 6.05 grams in 4 minutes (1.5 grams per minute)

12.36 p.m. 5th specimen, 0.55 gram in 30 seconds (1.1 grams per minute). 6th specimen, 5.9 grams in 6 minutes (1.0 gram per minute)

Now obtained more indifferent blood from abdominal aorta.

Left adrenal weighed 0.491 gram and contained 0.58 mgm. epinephrin

The epinephrin assay of the adrenal vein blood (a few of the tracings are reproduced in fig. 2) gave no indication that any epinephrin whatever was present, although the segments used for the assay were more than usually sensitive. We have shown elsewhere (3) that strychnine causes a marked and sustained increase in the rate of liberation of epinephrin, doubtless through the nervous mechanism. Yet neither before nor after strychnine could any epinephrin be detected in the adrenal vein blood. It was found that with indifferent blood made up to a concentration of 1:165,000,000 adrenalin and then diluted with three volumes of Ringer's solution, adrenalin was easily detected. Indifferent blood made up to a concentration of 1:330,000,000 adrenalin and then diluted with its own volume of Ringer produced a definite inhibition of the intestine segment. When the weight on the lever was increased the reaction became still more delicate, so that indifferent blood made up with adrenalin to a concentration of 1:500,000,000 and then diluted with an equal volume of Ringer's solution gave a distinct effect (observation 29, fig. 2), as did a concentration of 1:650,000,000 (observation 33). Even a concentration of 1:800,000,000 could be plainly detected (observation 35, fig. 2), while the 6th adrenal blood specimen similarly diluted gave no inhibition whatever (observation 31). Numerous additional observations (not reproduced) were made, but no evidence was obtained that any of the adrenal specimens contained epinephrin.

The output could not have been as much as 0.000001 mgm. per minute for the dog, or 0.0000002 mgm. per kilogram per minute, i.e. not $\frac{1}{1000}$ of the normal output. The section of the nerves was, therefore, complete. The failure of strychnine to excite any detectable secretion confirms this conclusion.

Although in these experiments the condition of the animals at the time they were sacrificed for the determination of the residual output of epinephrin was so good that there could be no reasonable doubt that they would have survived indefinitely, the next dog was allowed

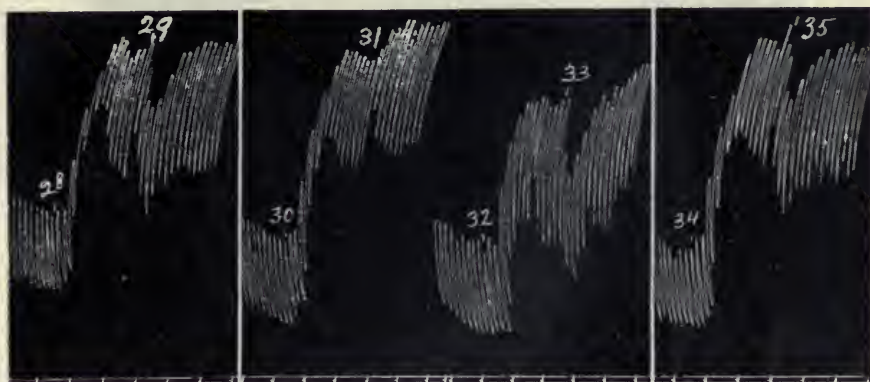


Fig. 2. Intestine tracings. Bloods from dog 242. At 28, 30, 32 and 34 Ringer was replaced by indifferent blood to which was added adrenalin to make a concentration of 1: 500,000,000; at 31 by the 6th adrenal specimen; at 33 by indifferent blood to which was added adrenalin to make a concentration of 1: 650,000,000 and at 35 by indifferent blood to which was added adrenalin to make a concentration of 1: 800,000,000. All the bloods were diluted with one volume Ringer (the adrenalin bloods after adding the adrenalin). (Reduced to one-half.)

to live for a longer period (eighty days). For the determination of the degree in which the epinephrin output has been suppressed by the operation this longer survival period has one disadvantage, namely, the possibility that regeneration of the secretory fibers may have occurred before the residual output of epinephrin is estimated.

CONDENSED PROTOCOL

Dog 243. Male. Weight 6.5 kgm.

October 28, 1918. Right adrenal excised. It weighed 0.428 gram. Nerves of left adrenal cut as in dog 241. The animal made a rapid recovery.

January 16, 1919. Condition excellent. Weight 7.6 kgm.

Erratum

Vol. xlviii, page 401. Legend of figure 2, line 2, after "blood" insert 'and this at 29 by indifferent blood.'

Under morphine and ether obtained a sample of jugular blood. Then made cava pocket as in the other dogs, and collected adrenal blood.

12.45 p.m. 1st specimen, 2.05 grams in 30 seconds (4.1 grams per minute). 2nd specimen, 8.35 grams in 2 minutes (4.1 grams per minute)

12.50 p.m. Injected 0.5 mgm. strychnine sulphate intravenously. Tonic convulsions. Artificial respiration started. Collected adrenal blood.

12.50½ p.m. 3rd specimen, 1.15 grams in 30 seconds (2.3 grams per minute). 4th specimen, 5.5 grams in 2 minutes (2.75 grams per minute)

1.05 p.m. Convulsions ceased; spontaneous respiration going on, with hyperpnoea. Stopped artificial respiration.

1.15 p.m. Reflexes still exaggerated. Collected adrenal blood. 5th specimen, 2.5 grams in 45 seconds (3.3 grams per minute). 6th specimen, 6.9 grams in 2 minutes (3.45 grams per minute)

Now obtained blood from abdominal aorta. Left adrenal weighed 0.49 gram.

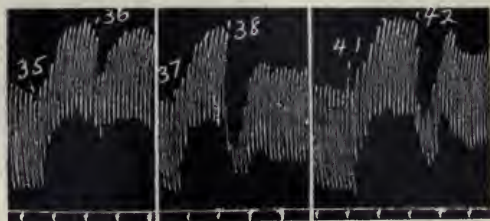


Fig. 3. Intestine tracings. Bloods from dog 243. At 35, 37 and 41 Ringer was replaced by jugular blood and this at 36 by jugular blood to which was added adrenalin to make a concentration of 1:10,000,000; at 38 by jugular blood to which was added adrenalin to make a concentration of 1:8,000,000; at 42 by the 2nd adrenal specimen. All of the bloods were diluted with one volume Ringer (the adrenalin bloods after adding the adrenalin). (Reduced to one-half.)

The epinephrin assay showed that the 2nd adrenal specimen had a concentration less than 1:7,000,000 and greater than 1:10,000,000 (fig. 3, observations 36 and 42—confirmed by several pairs of observations not reproduced). Observation 38 (fig. 3) indicates that the 2nd specimen was somewhat less than 1:8,000,000. The 4th specimen (taken after strychnine injection) had a much greater concentration than 1:7,000,000 and was stronger than 1:4,000,000 (fig. 4, observations 44 to 56). Figure 5 (observations 58, 60 and 62) shows that the 4th specimen did not differ much from 1:2,800,000. It was decidedly stronger than 1:4,000,000 and much weaker than 1:1,300,000 (fig. 5, observation 64). Qualitatively the 4th specimen was found to be much stronger than the 2nd (intestine observations not reproduced, confirmed by uterus observations 80 and 81, fig. 6). The assay indicates that the difference between the 4th and 2nd specimens is more

than can be accounted for by the difference in the blood-flows. Taking the 2nd specimen at 1: 9,000,000, we get 0.00045 mgm. per minute for the dog, or 0.00006 mgm. per kilogram per minute, i.e., $\frac{1}{3}$ to $\frac{1}{4}$ of the

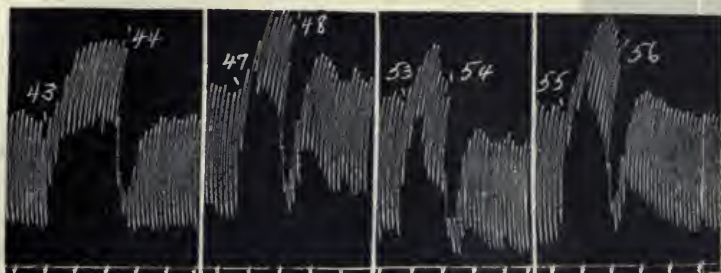


Fig. 4. Intestine tracings. Bloods from dog 243. At 43, 47, 53 and 55 Ringer was replaced by jugular blood, and this at 44 and 54 by the 4th adrenal specimen; at 48 by jugular blood to which was added adrenalin to make a concentration of 1: 7,000,000; at 56 by jugular blood to which was added adrenalin to make a concentration of 1: 4,000,000. All the bloods were diluted with one volume Ringer (the adrenalin bloods after adding the adrenalin). (Reduced to one-half.)

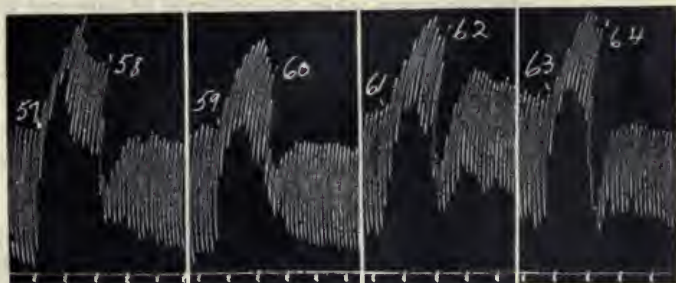


Fig. 5. Intestine tracings. Bloods from dog 243. At 57, 59, 61 and 63 Ringer was replaced by arterial blood and this at 58 by arterial blood to which was added adrenalin to make a concentration of 1: 2,800,000; at 60 by the 4th adrenal specimen; at 62 by arterial blood to which was added adrenalin to make a concentration of 1: 4,000,000; at 64 by arterial blood to which was added adrenalin to make a concentration of 1: 1,300,000. The bloods were diluted with one volume Ringer (the adrenalin bloods after adding the adrenalin). (Reduced to one-half.)

normal output for both adrenals. This would correspond to $\frac{1}{2}$ to $\frac{3}{4}$ the normal output for the denervated adrenal. As we have never found so great an output after this operation when the assay was made two or three weeks after the operation, the suggestion is that some

regeneration must have occurred. This conclusion is strengthened by the fact that strychnine seems to have increased the output. Thus, if the 4th specimen is taken at 1:2,800,000, the output per minute after strychnine would be 0.001 mgm. for the dog or 0.00013 mgm. per kilogram per minute, about double the initial output. The increase is not very great compared with that usually caused by strychnine in a normal animal, but this may be because regeneration was incomplete at the time the animal was sacrificed. In any case we should not venture to draw from this experiment the definite conclusion that regeneration of these fibers had occurred to a substantial extent in the eighty days since the operation. It is always possible in the present state of our knowledge of these secretory paths that owing to some anomaly a large portion of the fibers was spared by our operation. Our only ground for considering this to be improbable is that we have not encountered the condition when the interval of survival was only a few weeks.



Fig. 6. Uterus tracings. Bloods from dog 243. At 80 Ringer was replaced by the 4th adrenal specimen; at 81 by the 2nd adrenal specimen. The bloods were diluted with ten volumes Ringer. (Reduced to one-half.)

The normal output of epinephrin was estimated in two monkeys.

CONDENSED PROTOCOL

*Macacus cynomolgus.*² Female. Weight 2.51 kgm.

Under morphine and urethane a sample of blood was drawn from the internal jugular vein. Then the cava pocket was made as in the other experiments, the abdominal aorta being tied, but not the coeliac or mesenteric arteries. Adrenal blood was collected:

1st specimen, 3.4 grams in 1 minute (3.4 grams per minute)

2nd specimen, 7.0 grams in 3 minutes (2.33 grams per minute)

3rd specimen, 4.95 grams in 3 minutes (1.65 grams per minute)

More jugular and some arterial blood was now obtained. The right adrenal weighed 0.286 gram, the left 0.283 gram.

It was found at autopsy that a vein coming from the upper pole of the right adrenal passed up through the liver before entering the cava. The blood from this vein which, from its size, probably carried about one-third of the blood from

²In a previous paper this animal, an immature specimen showing complete permanent set of teeth with exception of the third molars above and below, was incorrectly referred to as a baboon.

that adrenal, would not be collected in clipping off the cava pocket. This vein was seen in the other monkeys operated on and must always be taken account of. The main adrenal vein from the right gland entered the cava at about the level usual in the dog and the blood from it could be collected.

It was shown on rabbit segments that the 3rd specimen was stronger than the 2nd, corresponding to the smaller flow; that the 2nd and 3rd specimens were stronger than 1:7,000,000 and weaker than 1:2,750,000 adrenalin; that the 3rd specimen was somewhat stronger than 1:4,800,000 and somewhat weaker than 1:4,000,000 while the 2nd specimen was somewhat weaker than 1:5,500,000.

Taking the 2nd specimen at 1:6,000,000 we get an output of 0.0004 mgm. per minute for the animal, or correcting for the loss of a portion of the blood from the right adrenal, say 0.0005 mgm., i.e., 0.0002 mgm. per kilogram of body weight per minute. Taking the 3rd specimen at 1:4,300,000, we get the same output.

In another monkey (*Macacus rhesus*) weighing 8.4 kilograms the output with an adrenal blood flow of 7.5 grams per minute was 0.0013 per minute for the animal, or 0.00015 mgm. per kilogram per minute. The right adrenal weighed 0.465 gram. The left adrenal weighed 0.48 gram.

In two monkeys (*Macacus rhesus*) the adrenal operation (excision of the right gland, denervation of the left) was performed in the usual manner. One of the animals was sacrificed after twenty-two days for estimation of any residual epinephrin output. It was at that time in excellent condition and, so far as could be made out, unaffected in any way by the loss of the epinephrin secretion. The other monkey was allowed to survive and is still alive and well five months after the operation. His weight is precisely the same as at the time of the operation. The condensed protocol of the monkey which was sacrificed follows:

CONDENSED PROTOCOL

Macacus rhesus. Male.

October 29, 1918. Right adrenal excised. It weighed 0.325 gram and contained 0.52 mgm. epinephrin. The left adrenal was denervated. Recovery was rapid, and the animal was active the next day.

November 20, 1918. Condition excellent. Weight 4.1 kgm.

Under urethane obtained indifferent (internal jugular) blood. Then made cava pocket as in the other animals. Collected adrenal blood.

1st specimen, 2.8 grams in 1 minute (2.8 grams per minute)

2nd specimen, 5.45 grams in 2 minutes (2.72 grams per minute)

3rd specimen, 7.05 grams in 3 minutes (2.35 grams per minute)

4th specimen, 5.75 grams in 3 minutes (1.91 grams per minute)

Now obtained more venous blood through cannula pushed into right heart through inferior cava and also some arterial blood. Left adrenal weighed 0.442 gram and contained 0.66 mgm. epinephrin.

With the rabbit intestine and uterus segments none of the adrenal specimens gave any reaction from which the presence of epinephrin could be deduced. Samples of the tracings are reproduced in figures 7 to 9. In figure 7 it is shown that the 4th specimen could not have



Fig. 7. Intestine tracings. Bloods from monkey, whose right adrenal was excised and left adrenal denervated 22 days prior to experiment. At 13 and 17 Ringer was replaced by venous blood and this at 14 by the 4th adrenal specimen; at 18 by venous blood to which was added adrenalin to make a concentration of 1: 130,000,000. The bloods were diluted with one volume Ringer (the adrenalin blood after adding the adrenalin). (Reduced to one-half.)

contained epinephrin even in a concentration of 1: 130,000,000. Even with the undiluted blood the 3rd specimen produced no definite inhibition of the intestine (fig. 8, observation 22). Another test with the 3rd specimen immediately after observation 30 gave an absolutely negative result. Yet the segment gave a distinct reaction with 1: 330,000,000 adrenalin in indifferent blood (observation 30), a stronger reaction with 1: 160,000,000 and a marked effect with 1: 80,000,000.

It was confirmed on the uterus that the adrenal specimens caused effects which did not differ sufficiently from those produced by indifferent blood to permit the conclusion that they contained epinephrin. Thus, the increase of tone produced by the 4th specimen was little greater than that produced by indifferent (arterial) blood (fig. 9, observations 33 and 34). The effect caused by the 3rd specimen was no greater than that caused by indifferent (venous) blood, although adrenalin in a concentration of 1: 80,000,000 in the indifferent blood gave a good rise of tone (fig. 9, observations 43 to 45).

Since our last paper (1) on this subject was published we have had the opportunity, in connection with other researches, of estimating the residual output of epinephrin after excision of one adrenal and denerva-

tion of the other in a considerable number of cats, which were allowed to survive long enough to recover completely from the effects of the operation. The results are combined in table 2, which supplements the similar data already published in tabular form in a previous paper (4).

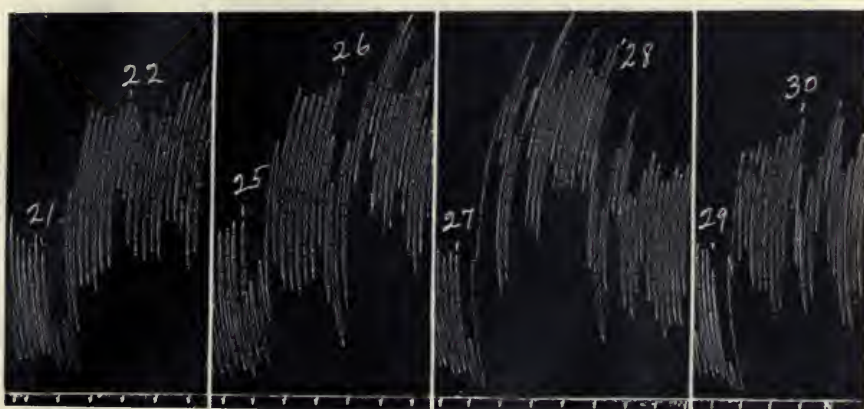


Fig. 8. Intestine tracings. Bloods from same monkey as in figure 7. At 21, 25, 27 and 29 Ringer was replaced by arterial blood and this at 22 by the 3rd adrenal specimen; at 26 by arterial blood to which was added adrenalin to make a concentration of 1: 160,000,000; at 28 by arterial blood with adrenalin to make a concentration of 1: 80,000,000; at 30 by arterial blood with adrenalin added to make a concentration of 1: 330,000,000. The bloods were not diluted before application to the segment. (Reduced to one-half.)

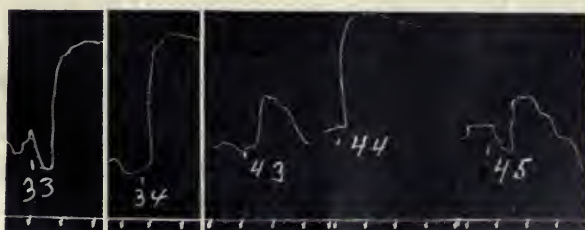


Fig. 9. Uterus tracings. Bloods from same monkey as in figures 7 and 8. At 33 Ringer was replaced by arterial blood; at 34 by the 4th adrenal specimen. The bloods were diluted with five volumes Ringer. With another uterus segment, at 43 Ringer was replaced by the 3rd adrenal specimen; at 44 by venous blood with adrenalin added to make a concentration of 1: 80,000,000, and at 45 by venous blood. The bloods were diluted with one volume Ringer (the adrenalin blood after adding the adrenalin). (Reduced to one-half.)

Five cats, not included in the table, were sacrificed in experiments which did not permit an assay of the adrenal blood. They had survived for a period of from ten to sixty-two days in good health.

Since it is important for estimating the degree in which the epinephrin output has been affected by denervation or by any other procedure, to establish on the basis of as large a number of observations as possible

TABLE 2

NUMBER OF CAT	BODY WEIGHT	EPINEPHRIN OUTPUT		FRACTION OF NORMAL LIBERATION	DAYS AFTER OPERATION
		Per minute	Per kilogram per minute		
	<i>kgm.</i>	<i>mgm.</i>	<i>mgm.</i>		
201	2.575	0.000012	0.0000045	$\frac{1}{50}$	18
202	2.545	0.000007	0.000003	$\frac{1}{100}$ *	8
215	2.38	0.00007	0.00003	$\frac{1}{10}$	59
219	2.54	0.000046	0.000018	$\frac{1}{12}$	27
231	3.08	0.00003	0.00001	$\frac{1}{25}$ *	33
233	2.62	0.000033	0.000013	$\frac{1}{20}$	36
235	2.25	0.000002	0.0000009	$\frac{1}{250}$ *	40
277	1.75	0.000055	0.00003	$\frac{1}{5}$ †	21
304	2.15	0.000033	0.000016	$\frac{1}{15}$	46

* In these cats there was no evidence that any epinephrin was being liberated. The amount given in the table is such as could have been detected by the test objects.

† Strychnine was administered before collection of the adrenal blood; this may have increased the output, so that the amount indicated by the assay is probably higher than the actual residual liberation.

In cat 215, assay by eye reactions gave an output of 0.00014 mgm. per minute for the animal, or 0.00006 mgm. per kgm. per minute, i.e., about $\frac{1}{5}$ of the normal average. In cat 233, assay by eye reactions gave an output of 0.00006 mgm. per minute for the animal, or 0.000023 mgm. per kilogram per minute, i.e., $\frac{1}{25}$ of the normal average.

the normal average output and the normal range, additional data on cats are collected in table 3. All the assays given in tables 1 to 3 were made on rabbit intestine (and uterus) segments.

The average output of epinephrin per kgm. of body weight per minute for the urethanized cats is 0.00025 mgm., the same as that given in the previous paper (1). The average for the 16 etherized cats is 0.0002 mgm. The average for the 45 cats included in table 2 of the previous paper and table 3 of this paper is 0.00023 mgm. per kgm. per minute.

TABLE 3

NUMBER OF CAT	BODY WEIGHT	WEIGHT OF ADRE- NALS	BLOOD FLOW				EPINEPHRIN CONCENTRATION	EPINEPHRIN	
			Grams	Min- utes	Seconds	Grams- per minute		Per animal per minute	Per kilogram per minute
	<i>kgm.</i>	<i>grams</i>						<i>mgm.</i>	<i>mgm.</i>
204	3.25	0.60	6.4		30	12.8	1: 14,000,000	0.0009	0.00027
205	2.7	0.68	7.3	3		2.4	1: 4,000,000	0.0006	0.0002
210	2.9	0.583	3.7	7		0.5	1: 800,000	0.0006	0.0002
211	2.8	0.58	3.5	4		0.9	1: 2,000,000	0.0005	0.0002
212	2.2	0.496	4.3	3		1.43	1: 3,200,000	0.00045	0.0002
213	2.67	0.468	9.8	3		3.3	1: 3,500,000	0.001	0.00037
214	2.35	0.348	7.0	3		2.3	1: 2,000,000	0.0011	0.00045
222	2.17	0.298	7.0	3		2.3	1: 5,500,000	0.0004	0.0002
223	3.47	0.462	6.1	2	30	2.45	1: 2,500,000	0.001	0.0003
225	2.05	0.292	3.7	3		1.2	1: 2,400,000	0.0005	0.00025
228	2.5	0.492	5.2	1	30	3.5	1: 5,800,000	0.0006	0.00024
229	2.07	0.387	4.7	3		1.6	1: 2,200,000	0.0007	0.00035
230	2.08	0.412	4.75	3		1.6	1: 2,500,000	0.0006	0.0003
238	1.64	0.288	5.3	3		1.7	1: 5,000,000	0.00034	0.0002
239	2.41	0.35	4.6	2		2.3	1: 5,000,000	0.00046	0.0002
258	2.76	0.323	6.05	3		2.0	1: 4,800,000	0.00042	0.00015
259	4.1	0.73	9.05	2		4.5	1: 4,500,000	0.001	0.00025
281	4.37	0.553	7.8	2		3.9	1: 4,500,000	0.00087	0.0002
282	3.1	0.51	5.7	2		2.9	1: 3,000,000	0.0009	0.0003
283	3.38	0.33	5.55	2		2.8	1: 7,000,000	0.0004	0.00012
284	2.85	0.502	4.0	2		2.0	1: 7,000,000	0.0003	0.0001
285	4.4	0.553	9.2	2		4.6	1: 6,500,000	0.0007	0.00016
286	4.0	0.372	7.1	1	30	4.7	1: 6,500,000	0.00072	0.00018
287	3.71	0.376	4.8	3		1.6	1: 6,500,000	0.00035	0.0001
288	2.175	0.37	4.2	2		2.1	1: 3,800,000	0.00055	0.00025
289	2.83	0.342	6.0	3		2.0	1: 1,300,000	0.0015	0.0005
290	2.265	0.296	3.3	3		1.1	1: 2,000,000	0.00055	0.00025
292	2.07	0.278	2.35	5		0.5	1: 1,300,000	0.0004	0.0002
293	3.865	0.377	8.8	1	30	5.87	1: 9,000,000	0.00065	0.00017
294	2.73	0.316	6.85	1	30	4.8	1: 6,600,000	0.0007	0.00025
295	2.9	0.372	6.45	2		3.2	1: 8,800,000	0.0005	0.00017
296	2.0	0.215	3.7	4		0.92	1: 2,300,000	0.0004	0.0002
298	3.35	0.418	5.35	3		1.8	1: 3,000,000	0.0006	0.00018
303	4.82	0.66	9.05	1	30	6.0	1: 6,000,000	0.001	0.0002
305	3.41	0.312	6.8	2		3.4	1: 6,000,000	0.00057	0.00017

The first 19 cats in the table (down to and including cat 282) were anesthetized with urethane, the rest with ether.

CONCLUSION

In dogs and monkeys, as previously shown for cats and rabbits, the liberation of epinephrin from the adrenals is not indispensable for life and health.

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A NOTE ON SOME OBVIOUS CONSEQUENCES OF THE HIGH RATE OF BLOOD FLOW THROUGH THE ADRENALS

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It has been shown by a number of observers that the rate of blood flow through the adrenal glands is exceptionally great. According to Neuman (1), it amounts in cats to 6 to 7 cc. of blood per minute per gram of organ, with a blood pressure of 130 mm. of mercury, a greater flow than that through any other organ.

Stewart and Rogoff (2) have published results on the rate of filling of a cava pocket from the adrenal veins in cats, from which the rate of flow through the glands with unopened blood vessels and in the absence of hemorrhage can be calculated. For example, in five successive observations while the circulation was fairly good, the blood flows were 2.8 cc., 2.6 cc., 2.2 cc., 2.3 cc., 2.5 cc. per gram of gland per minute. As the mesenteric and coeliac arteries were not tied and the arterial blood pressure was rather low in this experiment (38 to 42 mm. of mercury during the observations quoted), these rates of flow are doubtless below the average with blood pressures more nearly approaching the normal.

In other papers (3), we have published numerous observations in which the blood flow through the adrenals was estimated by collecting the blood through a cannula. In the great majority of these experiments the coeliac, mesenteric and renal arteries and the abdominal aorta were tied. Therefore the arterial pressure at the commencement of collection of the blood was generally high.

In twenty-three cats the blood flows in grams per minute per gram of gland were: 3.3, 4.2, 6.4, 11.3, 5.0, 7.0, 5.6, 4.5, 3.0, 1.4, 2.7, 3.2*, 7.9*, 6.0*, 4.5*, 4.2, 9.1*, 4.9*, 6.6*, 2.0*, 2.5*, 3.1, 6.0. Average, 5.0 grams.

The numbers marked with an asterisk are from animals in which only one adrenal remained, the other having been extirpated some weeks

previously. The average of these results shows practically the same flow as the average of the whole. In all the experiments several samples of blood were collected through the cannula. The rate of blood flow is usually calculated for the second sample, the first being rejected if much higher than the others because of the possibility of some blood being included in the cava pocket at the time it was clipped off, which would of course increase the apparent rate of outflow in the first sample. The results may accordingly be considered as certainly not too high for the given experimental conditions (ligation of alternative arterial paths, relatively high arterial pressure and almost zero pressure in the vein).

Burton-Opitz and Edwards (4), working with a stromuhr, obtained in dogs an average blood flow of 4.9 grams per gram of gland. This, as they point out, is very much more than the flow through any other organ with the possible exception of the thyroid. In connection with the figures given in the literature for the thyroid, it may be remarked that although there is no doubt that the blood flow through the normal thyroid is large in comparison with that of most organs, some of the experiments were probably performed on hyperplastic glands.

From the relatively great quantity of blood passing through the adrenals, it might be expected that the blood of the adrenal veins would differ less from arterial blood than ordinary venous blood does. It would hardly be credible, for instance, that the percentage loss of oxygen by the arterial blood in the adrenals should be as great as in the generality of tissues. It might, therefore, be looked for that adrenal vein blood should be richer in oxyhemoglobin than ordinary venous blood obtained, say, from the jugular vein.

If 5 grams of blood pass through a gram of adrenal in a minute, and the oxygen consumption of the gland is taken as 0.05 cc. (Neuman gives 0.045 cc.) per gram of organ per minute, the blood issuing from the adrenal veins would contain only 1 cc. of oxygen per 100 cc. of blood less than the arterial blood. In order that the difference should become as great as in ordinary mixed venous blood, say 8 volumes of oxygen per cent, the consumption of oxygen would have to rise to 0.4 cc. per gram of adrenal substance per minute. This would be five times as great as the oxygen consumption given by Barcroft for striated muscle during maximal activity. Yet a recent writer (5) thinks it worth while to demonstrate that the oxyhemoglobin bands in a dilution of adrenal vein blood are stronger than the oxyhemoglobin bands in jugular vein blood from the same animal, diluted to the same degree with oxygen-

free saline solutions, and to seek for an explanation of this fact in some action exerted by adrenalin in "augmenting the oxygen-absorbing capacity of hemoglobin."

The facts that adrenal vein blood often "develops an arterial appearance" in the vein when the latter is clamped at its outlet into the vena cava, and that on dilution with salt solution it becomes red sooner than ordinary venous blood are easily understood as soon as it is recognized that adrenal vein blood is nearer to arterial blood than ordinary venous blood. There is no evidence and no likelihood that adrenalin has anything to do with the matter at all.

"The observation that the addition of adrenalin to the perfusion fluid favorably affects the oxygen intake of the heart in perfusion experiments" ought not, I think, to suggest "that the base may play a similar rôle in relation to the oxygen-absorption of hemoglobin in the adrenal vein." For anything which strengthens the action of the heart may be expected to increase its oxygen consumption.

Again, it is known that the H-ion concentration of ordinary venous blood is somewhat greater than that of arterial blood. It might be expected from the great blood flow through the adrenal gland that the adrenal vein blood would be somewhat more alkaline than ordinary venous blood since it is nearer to arterial blood, and in particular cannot be supposed to have acquired as much carbon dioxide in its passage through the glands as mixed venous blood. The realization of this would probably have modified the statement in another paper (6)¹ that "the increased alkalinity (in the adrenal vein blood) is due to the dissolved adrenalin which it contains."

It is not easy to gather in what way these writers suppose that the change in the reaction of the adrenal vein blood is produced by the adrenalin in it. It is scarcely necessary to point out that it is highly improbable that the mere addition of the base adrenalin in the concentration of a $\frac{1}{500,000}$ molecular solution (which would correspond to a normal concentration of adrenalin in adrenal vein blood) to a liquid with the buffer properties of blood could produce an effect on the H-ion concentration measurable by the gas-chain method.

¹ The paper referred to, although headed "From the Cushing Laboratory for Experimental Medicine, Western Reserve University," was not seen by me until recently. The work was not done under my direction.

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THE RELATION OF THE EPINEPHRIN OUTPUT OF THE ADRENALS TO CHANGES IN THE RATE OF THE DENERVATED HEART

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INTRODUCTION

It is well known that adrenalin causes acceleration of the heart after section of the vagi and excision of the stellate ganglia. v. Anrep (1) showed in the dog that stimulation of the peripheral end of the splanchnic nerve caused acceleration, associated with the peculiar features of the splanchnic blood pressure curve which Elliott (2) explains as due to the augmented epinephrin output known to be elicited by stimulation of that nerve. Pearlman and Vincent (3), working with the heart only partially isolated from the central nervous system by section of the vagi, have confirmed Elliott's interpretation of the peculiar form of the blood pressure curve caused by splanchnic stimulation and observed manifest augmentation of the heart. We have ourselves shown (in the cat) that the epinephrin given off at the normal average rate under the conditions of our experiments causes acceleration when the adrenal vein blood collected in a cava pocket for a minute or two is released. The acceleration begins a little time after release of the pocket, the length of the interval depending upon the state of the circulation, and is coincident or nearly so with the dilatation of the pupil if the superior cervical ganglion has been previously excised. The beginning of the dilatation has been seen to coincide approximately with the "dip" associated by Elliott with the action of the liberated epinephrin (4). Also when the splanchnic is stimulated with the adrenal veins open it can sometimes be seen that about the same time as the reactions of the eye (sensitized by removal of the superior cervical ganglion) appear, the denervated heart begins to become accelerated. From all this it seems clear that epinephrin

liberated from the adrenal in response to stimulation of the splanchnic plays a part in the acceleration of the heart. We shall show later on that the whole acceleration is not due to the epinephrin, but that other factors are involved, since a good acceleration, even as great as that obtained with intact adrenals, can be observed when they have been removed or when discharge of epinephrin has been prevented in other ways. It will be pointed out in discussing these results that, with such a reaction as acceleration of the heart, there is no inconsistency in attributing a share in the reaction to epinephrin and yet asserting that sometimes as great a maximum acceleration may be attained in its absence as when it is being given off. It is possible that the position of the maximum acceleration or of the beginning of the acceleration on the blood pressure curve may be shifted when epinephrin action is excluded. But however this may be, it will, we believe, be evident when our results have been displayed that it would be a very unpromising venture to attempt to found a method of estimating the rate of output of epinephrin upon such a reaction, even under conditions, as in splanchnic stimulation, in which it is known that an increase in the epinephrin output can contribute to it. Where adrenal blood is collected in a cava pocket and then released there is no question that the acceleration is produced by the epinephrin and practically by that alone, because the experiment has been so simplified that only one factor is acting, namely the admission into the circulation of the epinephrin-containing blood. But even here the reaction is of such a character that it could hardly lend itself to anything like an exact assay of the epinephrin in the blood from the pocket.

Nevertheless in a recent paper Cannon (5) has described experiments on cats in which, from the acceleration of the so-called denervated heart caused by stimulation of the central end of the sciatic nerve, by asphyxia and by emotional excitement, he professes to prove that these conditions produce a marked increase in the rate of output of epinephrin from the adrenals. Apparently admitting that the catheter method (6) is a difficult one to obtain positive results with, he introduces this as a relatively simple method which can be carried out by "any competent experimenter," and he states that the results of these experiments confirm "in every particular" his previous conclusions as to the influence of emotional excitement, asphyxia and sensory stimulation upon the adrenal secretion. We have never quarreled with the catheter method because of its difficulty, but because it cannot yield the data necessary to determine the rate of output of epinephrin or to

measure the changes in that rate. We shall take another occasion to point out again the reasons why we cannot accept Cannon's conclusions based on experiments with the catheter method. The technique of obtaining the cava blood is surely not beyond the reach of "any competent experimenter" in physiology, and the assaying of the epinephrin in the blood would be easy if such concentrations existed there as, by implication, we must conclude that Cannon assumed to exist during or after the action of the factors studied by him.

The denervated heart reaction now adopted by Cannon "as an indicator of adrenal secretion" is also easy enough to carry out. But it labors under even more serious defects, when employed as a quantitative method of measuring the epinephrin output and of estimating changes in the rate of output, than does the catheter method. For in the latter an attempt was at any rate made to obtain blood containing a portion of the epinephrin given off by the adrenals and to test it by a method of bio-assay which, if properly applied, does permit the epinephrin concentration in the sample of blood to be estimated. When Doctor Cannon stimulates the central end of the sciatic numerous reflex effects may be caused, the consequences of which upon the rate of the heart cannot be easily controlled. Among these the vasomotor reflex changes are conspicuous, and it is obvious that in this way great variations may be produced in the pressure in the cavities of the heart, the rate of blood flow through the coronary system and, therefore, the amount of epinephrin passing through the coronaries, any of which may lead to an acceleration of the heart beat without any change having occurred in the rate of output of epinephrin.

Cannon, however, states that after removal of the adrenals or their ligation *en masse* or after removal of one adrenal and section of the splanchnic of the other side, acceleration of the heart is no longer caused by sciatic stimulation. In one experiment he obtained some acceleration after division of both splanchnics in the thorax, but much less than before. He concludes that the acceleration following sciatic stimulation is due entirely to a reflexly increased output of epinephrin. And obviously assuming that no other factors are involved, he states that "comparisons of the increased rate due to sciatic stimulation with effects of adrenalin (quantitated as base) injected intravenously indicate that the range of reflex adrenal secretion lies between 0.001 and 0.005 mgm. per kgm. per minute, i.e., from 5 to 25 times the amount regarded by Stewart and Rogoff as the normal output." In view of our own results proving conclusively that epinephrin, if a factor, cannot

be the sole factor in the heart reaction elicited by stimulation of the sciatic, it would scarcely be worth while to spend much time in examining such data. But it may be pointed out that Cannon does not state by what control experiments he has established a quantitative relation between the *maximum* acceleration reached and the dose of adrenalin, and not, for instance, between the dose of adrenalin and the duration of the acceleration or the total surplus number of beats in the period of acceleration. Also if such data are to have real quantitative value the adrenalin ought to be administered while the blood pressure and therefore the coronary blood flow are increased to approximately the same extent as during stimulation of the sciatic.

As regards Cannon's statement that the reaction is abolished by removal of the adrenals, it must be noted that, if it were granted that the only change caused by removal of the adrenals or section of the splanchnics is the suppression of the epinephrin output, his result would simply show that the acceleration previously obtained had been due essentially to epinephrin. It would not show that any increase had occurred in the rate of output, unless it were demonstrated that a redistribution of the blood, due to the vascular reactions evoked by the stimulation and necessarily associated with the passage through the coronary circulation of an increased proportion of the epinephrin already being given off, was insufficient to account for the reaction.

But it cannot be granted that the operations practised to eliminate the epinephrin output have no other consequences. It is astonishing with what indifference both splanchnics are cut merely in order to interfere with the epinephrin output, as if all or most of the splanchnic fibers innervated the adrenals. The same is true of the removal of the adrenals. Some writers seem to assume that it is practically impossible to injure any important nerves when the adrenals are removed or tied off and that all the consequences which follow their removal are necessarily due to the loss of epinephrin. Gley and Quinquaud (7) have pointed out that the opposite is the case. In the dog according to them it is practically impossible to remove the adrenals without severely injuring the splanchnics. They say, however, that with their large experience they have succeeded in operating on the dog also, so as to eliminate the adrenals without injury to the splanchnics. Pearlman and Vincent (3) take exception to Gley's statement that the difference in the splanchnic blood pressure curve observed by v. Anrep (1) in dogs after and before removal of the adrenals is due to injury to nerve fibers, and no doubt in Vincent's hands the operation is as little harmful as it

is possible for it to be. If the operation can be more easily done in the cat, it still needs care and experience to reduce this cause of error to a minimum, particularly in the case of the right adrenal, the ligation of which is liable to injure the splanchnic. For this and other reasons the removal of the adrenals, as frequently done in acute experiments, may be attended with a marked drop in the blood pressure not related to loss of function of the glands. It must be remembered that in experiments on the denervated heart, the adrenals are removed after a considerable preliminary operation and the extrinsic regulatory nerves of the heart are eliminated. This may make it more difficult to excise or tie off the adrenals without a fall of blood pressure than in experiments on normal animals, such as those of Young and Lehman (8), of Hoskins and McClure (9) and of Bazett (10). The diminution in the heart rate remarked by Cannon, and apparently attributed by him entirely to the loss of epinephrin, is according to our observations not unrelated to the fall of pressure, although since there is reason to believe that the epinephrin liberated at the ordinary rate may affect the heart, some part of the slowing may be due to loss of epinephrin. Cannon has not given any data by which one can judge how great the change of blood pressure was in his experiments, but as he injected gum salt solution in one of them the fall may be assumed to have been sometimes considerable. Now, whatever interpretation one chooses to put upon the heart acceleration produced by sciatic stimulation, a reflex or more than one reflex action must be essentially involved in it. Any operation which impairs the conductivity of the reflex arcs must, therefore, tend to diminish or abolish the effect. And if a negative result obtained after removal of a certain organ is attributed solely to the specific effect which the operator intended to produce, and not at all to the general effects which he did produce, then, of course, positive results elicited before the operation in any way whatever will seem to depend entirely upon the specific activity of the organ removed.

In what has been said above we desire to state distinctly that we do not imply that Doctor Cannon did not remove the adrenals skilfully, and with full knowledge of the importance for his control experiment of injuring the nerves in the vicinity as little as possible and of maintaining the animal in a good general condition. All we know is that we obtained positive results after elimination of the adrenals where he obtained completely negative results. It is clearly the positive results which are significant for the decision of the question of the relation of

the epinephrin output to the heart acceleration caused by sciatic stimulation and not the negative ones.

Our experiments were all made on cats. The greater number of them were performed two years ago. Only a brief notice of a portion of them has been published (11). They were mentioned, so far as could be done in the few minutes allotted to us, in the discussion of Doctor Cannon's paper at the meeting of the American Physiological Society last December. We studied the effect upon the acceleration produced by stimulation of sensory nerves of eliminating the epinephrin secretion:

a. By clipping the adrenal veins, either with or without simultaneous ligation of the renal vessels.

b. By removing one adrenal (the right) and denervating the other and allowing the animal to recover completely from the operation. In a number of these animals the denervated adrenal was also removed in the final experiment, and the heart reactions obtained before and after its removal compared.

c. By removing one adrenal (almost always the right) and allowing an interval for the animal to recover, before performing the experiment upon sensory nerve stimulation with removal of the other adrenal. In this way it was supposed that the condition of the animal after removal of the remaining adrenal would be better than if both were removed at the time of the experiment.

d. By removing both adrenals during the experiment on the heart reaction. In all cases in excising the glands every precaution was taken to avoid injury to nerves, by making a careful dissection between the capsule of the gland and the cortex, tying the vessels with fine ligatures.

EXPERIMENTS IN WHICH THE ADRENAL VEINS WERE CLIPPED OFF OR TIED

In principle this is the most satisfactory way of eliminating the epinephrin output, since no other organ than the adrenals is interfered with and there is no damage to important nerves and no injurious fall of blood pressure. The procedure has been extensively employed by Gley. v. Anrep (1) also used it in some of his experiments on splanchnic stimulation and satisfied himself that the effects attributed by him to epinephrin were not obtained when the suprarenal vein was clipped and the corresponding splanchnic stimulated. And Pearlman and Vincent (3) state that they have usually obtained quite satisfactory

results by simply clamping and unclamping the adrenal veins. Cannon takes exception to clipping because it does not eliminate the heart reaction to sciatic stimulation or asphyxia which he interprets as indicating increased epinephrin output, and he, therefore, assumes that there must be leakage of epinephrin by anastomotic venous channels. He quotes, for instance, an experiment in which before ligation of the adrenal veins asphyxia of a certain duration caused an acceleration in the heart rate of 40 beats. After ligation of the veins the acceleration was precisely the same. We should have thought the only possible interpretation of such a result would have been that epinephrin had nothing to do with the reaction, in the particular experiment, at any rate. For who will believe that after the adrenal veins were tied just as much epinephrin passed out of the adrenals by some difficult collateral path, in the minute for which asphyxia was maintained, as would have passed out by the adrenal veins plus these hypothetical anastomotic channels? And how can the heart acceleration be a quantitative reaction for epinephrin if blocking the adrenal veins does not at least diminish the reaction? When a reaction known to be caused by epinephrin is studied the result is quite different. For example, we sometimes observe a small dilatation of the pupil (after previous removal of the superior cervical ganglion) on stimulation of the peripheral end of a splanchnic nerve, with the corresponding or even with both adrenal veins clipped. But this is much smaller than the reaction obtained before or after by similar stimulation with the veins open, and usually on release of the clips there is an additional and greater reaction indicating that epinephrin had been pent up in the adrenal veins by the clips. We suggest that the reason why Cannon gets such positive results with the adrenal veins tied, and must have recourse to removal or ligation of the adrenals to obtain negative results, is that in tying the veins he does not inflict such injury as causes a general deterioration of the animal incompatible with a positive heart reaction, whereas in removing the adrenals he appears to do so.

In our experiments with clipping of the adrenal veins we compared the heart acceleration and rise of blood pressure caused by stimulation of the sciatic when the blood flow from the glands and, therefore, the epinephrin output were going on unhindered, with the acceleration and blood pressure rise obtained when the flow from one or both adrenals was obstructed. It was supposed that if the epinephrin is an important factor in the heart reaction, the reaction would be distinctly

smaller with the adrenal veins clipped off, that is to say, provided that the reaction can be used at all as a quantitative test for epinephrin. The protocols show that this expectation was not realized. If the epinephrin liberated from the adrenals is an appreciable factor, it is not easy to disentangle its influence from that of the other factors which can affect the heart rate. It must be remembered that even if it were clearly demonstrated that the acceleration caused by sciatic stimulation was diminished by interference with the output of epinephrin, the other potential factors, such as rise of blood pressure, not being interfered with, this would only prove that epinephrin takes a share in the reaction, not that its rate of output is increased by the stimulation.

The peripheral end of a splanchnic nerve was also stimulated with the corresponding, or both adrenal veins clipped or open, in order to compare the effect of a procedure which is known to increase the epinephrin output on the heart rate with the effect of sciatic stimulation.

It will be seen that it may be difficult to demonstrate, by comparing the maximum acceleration caused with the adrenal veins clipped and open, that the epinephrin undoubtedly liberated by stimulation of the splanchnic takes any sensible share in the heart reaction. However, this is rather an illustration of the deficiencies of such a reaction as a quantitative test for epinephrin than a proof that the epinephrin liberated with the adrenal veins open is without effect upon the rate of the heart. As already mentioned, when the epinephrin is allowed to accumulate in a cava pocket or even when the epinephrin pent up in the adrenal vessels by clipping of the adrenal veins is released a distinct acceleration is produced, and it may be assumed that the epinephrin coming steadily off from the adrenals, without being accumulated, will tend to exert a similar action, especially when the amount of epinephrin passing per unit of time through the coronaries, or its concentration, is abruptly increased by the vasomotor changes associated with splanchnic or sciatic stimulation. It seems, however, improbable that with such a reaction as acceleration of the heart the acceleration produced by simultaneous action of two factors, singly effective, should be the sum of the separate effects, whether the reaction is measured by the maximum acceleration attained, or by the duration of the acceleration or by the total surplus number of beats. It seems more likely that when the heart is keyed up to a certain point by the action of one factor, whether this acts upon a local accelerator mechanism or not, it will break loose, so to speak, from the relative stability of rate

imposed upon it by removal of its extrinsic nerves, and execute a run of quicker beats, the duration and maximum acceleration of which may have no simple relation to the absolute magnitude of the exciting influence, and which may not be greatly modified by a concomitantly acting influence, itself capable of independently producing a similar reaction. The results of these experiments are illustrated by some protocols. Where it is mentioned that a nerve was stimulated with a vein clipped, the clip was applied, unless otherwise stated, a few seconds before the stimulation and was removed as soon as the portion of the curve to be used for counting the heart rate had been completed. When it is simply mentioned that a nerve was stimulated, without any reference to clipping, it is implied that the veins were open. Under "rate" is always given the number of heart beats per minute; under "pressure" the blood pressure in millimeters of mercury.

Protocol. Cat 175; male; weight, 2.2 kgm.

11:30 a.m. Urethane, 5 gm. by stomach tube.

1:10-1:48 p.m. Cut vago-sympathetics and excised stellate ganglia;¹ prepared central end of left sciatic for stimulation.

	<i>Rate</i>
1:50 p.m. Before stimulation of sciatic.....	188
25 seconds after beginning stimulation.....	210
1:55 p.m. Before stimulation of sciatic.....	187
20 seconds after beginning stimulation.....	202
40 seconds after beginning stimulation.....	212
Now opened abdomen and purposely manipulated intestines	
2:03 p.m. Before stimulation of sciatic.....	178
30 seconds after beginning stimulation.....	212
2:10 p.m. Before stimulation of sciatic.....	186
30 seconds after beginning stimulation.....	214
2:14 p.m. Before stimulation of sciatic; left adrenal vein clipped.....	184
30 seconds after beginning stimulation.....	206
2:16 p.m. Prepared peripheral end of left splanchnic in abdomen	
2:20 p.m. Before stimulation of splanchnic; left adrenal vein clipped....	184
15 seconds after beginning stimulation.....	192
30 seconds after beginning stimulation.....	208
45 seconds after beginning stimulation.....	224

¹ With the cat in the supine position an incision is made in the axilla and a part of the second rib exposed by separation of overlying muscle. A portion of the rib anterior to the angle is carefully separated from its periosteum and resected. The lower part of the stellate ganglion is usually thus exposed and the excision of the ganglion easily completed without injury to the pleura.

In all the experiments, except when otherwise stated, the period of stimulation of the sciatic was 30 to 40 seconds. When the heart rate is given after a certain number of seconds from beginning of stimulation this means that a sufficient portion of the curve beginning at that point was counted. The corresponding blood pressure was that at the end of the portion counted.

2:26 p.m.	Before stimulation of splanchnic; left adrenal vein clipped....	180	
	15 seconds after beginning stimulation.....	196	
	30 seconds after beginning stimulation.....	208	
	45 seconds after beginning stimulation.....	220	
	60 seconds after beginning stimulation.....	220	
2:32 p.m.	Before stimulation of splanchnic.....	182	
	During first 15 seconds of stimulation.....	180	
	During second 15 seconds of stimulation.....	200	
	During third 15 seconds of stimulation.....	204	
	During fourth 15 seconds of stimulation.....	216	
2:47 p.m.	Before stimulation of sciatic; both adrenal veins clipped.....	184	
	During first 15 seconds of stimulation.....	188	
	During second 15 seconds of stimulation.....	188	
	During third 15 seconds of stimulation.....	200	
	During fourth 15 seconds of stimulation.....	204	
2:53 p.m.	Before stimulation of sciatic.....	176	
	During first 15 seconds of stimulation.....	180	
	During second 15 seconds of stimulation.....	196	
	During third 15 seconds of stimulation.....	208	
	During fourth 15 seconds of stimulation.....	216	
3:05 p.m.	Before stimulation of sciatic; both adrenal veins clipped.....	188	
	During first 15 seconds of stimulation.....	188	
	During second 15 seconds of stimulation.....	196	
	During third 15 seconds of stimulation.....	204	
	During fourth 15 seconds of stimulation.....	204	
		<i>Rate</i>	<i>Pressure</i>
3:45 p.m.	Before stimulation of sciatic.....	176	82
	24 seconds after beginning stimulation.....	203	140
3:50 p.m.	Before stimulation of splanchnic; left adrenal vein clipped.....	174	58
	28 seconds after beginning stimulation.....	215	166
3:57 p.m.	Before stimulation of splanchnic; both adrenal veins clipped.....	185	62
	24 seconds after beginning stimulation.....	206	131
	40 seconds after beginning stimulation.....	221	
4:03 p.m.	Before stimulation of sciatic.....	174	46
	12 seconds after beginning stimulation.....	197	118
	40 seconds after beginning stimulation.....	200	
4:10 p.m.	Before stimulation of sciatic; both adrenal veins clipped.....	189	52
	15 seconds after beginning stimulation.....	200	112
	30 seconds after beginning stimulation.....	204	
4:15 p.m.	Before stimulation of splanchnic.....	184	55
	20 seconds after beginning stimulation.....	209	130
	Excised left adrenal		
4:30 p.m.	Before stimulation of splanchnic.....	215	70
	22 seconds after beginning stimulation.....	229	90

Some of the curves from these experiments are used in the next paper to demonstrate that the acceleration of the heart on stimulation of the central end of the sciatic, contrary to Cannon's statement, is easily obtainable after the abdomen has been opened. The protocols themselves and other protocols coming later in the present paper also afford abundant evidence that the statement is baseless. In figure 1 portions of the blood pressure curve from cat 175 before and during stimulation of the left splanchnic (*A* and *B*, taken at 3:50 p.m.) are

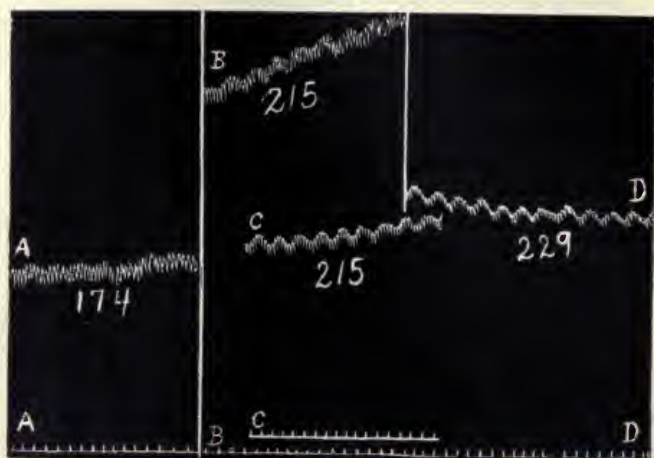


Fig. 1. Parts of blood pressure tracings from cat 175. *A*, before and *B*, a portion commencing 28 seconds after beginning of splanchnic stimulation with corresponding adrenal vein clipped; *C*, before and *D*, 22 seconds after beginning of splanchnic stimulation with corresponding adrenal excised. In all figures line of zero pressure corresponds with time trace; time in seconds; numbers above time trace represent heart rate per minute. Reduced to four-fifths.

reproduced, and corresponding portions after removal of the adrenal (*C* and *D*, taken at 4:30 p.m.). The maximum acceleration in *B* was 41 beats per minute; the maximum in *D*, 12 to 14 beats as compared with a maximum acceleration of 25 beats in the curve taken with the last splanchnic stimulation prior to removal of the adrenal. It is impossible to know how much, if any part, of the difference is due to the lack of epinephrin from the left adrenal when the curve *C D* was being written, since the stimulation was much less effective in raising the blood pressure, as shown in figure 2. The much reduced curves (black on white) are not intended for counting.

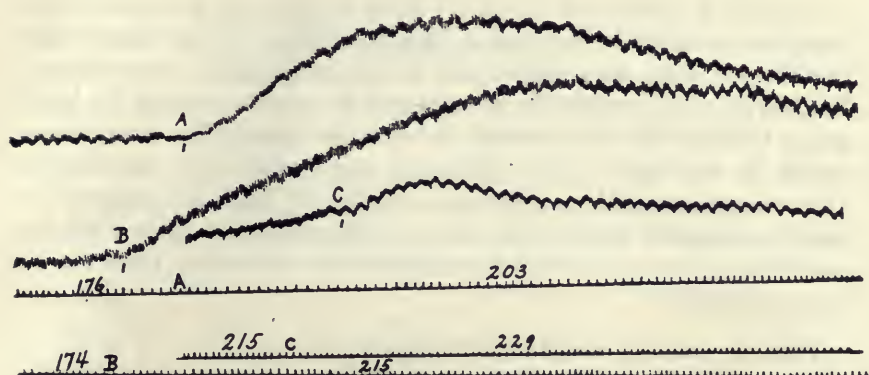


Fig. 2. Blood pressure curves from cat 175. *A*, sciatic stimulation; *B*, splanchnic stimulation with corresponding adrenal vein clipped; *C*, splanchnic stimulation with corresponding adrenal excised, 40 minutes after *B*. Reduced to one-half.

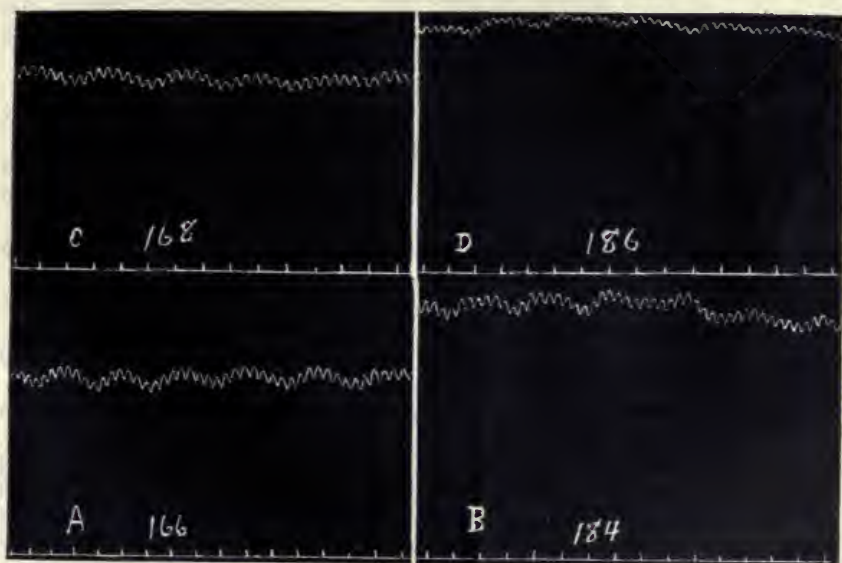


Fig. 3. Parts of blood pressure tracings from cat 237. *A*, before and *B*, a portion commencing 33 seconds after beginning of sciatic stimulation; *C*, before and *D*, 40 seconds after beginning of sciatic stimulation with both adrenal veins clipped. Reduced to three-fourths.

In figure 3 portions of the curve from cat 237 are given, *A* and *B* taken respectively before and during stimulation of the sciatic with the adrenal veins open and *C* and *D* with the adrenal veins clipped. The curves were practically parallel and the acceleration of the heart about the same in both (about 18 beats per minute), in the portions shown in the figure. The maximum acceleration with stimulation after clipping was 21, and without clipping 20 beats per minute. It may be remarked that not only had the abdomen been opened but the renal arteries and veins had been tied 40 minutes before these curves were obtained.

Protocol. Cat 177; male; weight, 3.6 kgm.

		Rate	Pressure
2:05 p.m.	Under urethane (6 gm.) cut vago-sympathetics; excised stellate ganglia and prepared central end of left sciatic for stimulation.		
2:25 p.m.	Before sciatic stimulation.....	185	132
	20 seconds after beginning stimulation.....	228	154
2:30 p.m.	Opened abdomen; prepared peripheral end of left splanchnic		
2:32 p.m.	Before sciatic stimulation.....	173	105
	24 seconds after beginning stimulation.....	200	126
	49 seconds after beginning stimulation.....	216	
2:35 p.m.	Before stimulation of left splanchnic; left adrenal vein clipped.....	167	108
	12 seconds after beginning stimulation.....	191	
	24 seconds after beginning stimulation.....	215	140
	45 seconds after beginning stimulation.....	211	110
2:40 p.m.	Before stimulation of sciatic; both adrenal veins clipped.....	181	80
	23 seconds after beginning stimulation.....	190	
	37 seconds after beginning stimulation.....	197	106
2:45 p.m.	Before stimulation of left splanchnic; left adrenal vein clipped.....	171	90
	17 seconds after beginning stimulation.....	194	
	22 seconds after beginning stimulation.....	206	124
2:50 p.m.	Before stimulation of left splanchnic; both adrenal veins clipped.....	191	100
	20 seconds after beginning stimulation.....	213	128
2:55 p.m.	Before stimulation of sciatic; left adrenal vein clipped..	184	94
	28 seconds after beginning of stimulation.....	192	107
3:00 p.m.	Before stimulation of sciatic; both adrenal veins clipped.....	167	78
	23 seconds after beginning stimulation.....	173	98
3:10 p.m.	Before stimulation of left splanchnic; left adrenal vein clipped.....	160	74
	18 seconds after beginning stimulation.....	194	92

3:15 p.m.	Before stimulation of left splanchnic; both adrenal veins clipped.....	160	82
	23 seconds after beginning stimulation.....	167	
	42 seconds after beginning stimulation.....	185	
	55 seconds after beginning stimulation.....	200	106
3:20 p.m.	Before stimulation of left splanchnic; left adrenal vein clipped.....	165	72
	20 seconds after beginning stimulation.....	183	82
3:30 p.m.	Before stimulation of left splanchnic; both adrenal veins clipped.....	166	68
	25 seconds after beginning stimulation.....	186	86
3:35 p.m.	Before stimulation of left splanchnic; left adrenal vein clipped.....	173	76
	20 seconds after beginning stimulation.....	180	90
4:10 p.m.	Before stimulation of peripheral end of right splanchnic in thorax, with left adrenal vein clipped.....	170	30
	15 seconds after beginning stimulation.....	205	78

In cat 177, in which a good acceleration of the heart was obtained with sciatic stimulation after opening the abdomen and section of one splanchnic nerve, the reaction from the sciatic after repeated splanchnic and sciatic stimulations and repeated clipping of the adrenal veins was distinctly diminished toward the end of the experiment. Stimulation of the peripheral end of the splanchnic continued to give a good acceleration of the heart when the reaction, as reflexly elicited by stimulation of the central end of the sciatic, together with the reflex rise of blood pressure, was being exhausted.

In comparing the acceleration accompanying a given rise of blood pressure caused by sciatic, with that accompanying a similar rise of pressure caused by splanchnic stimulation it generally appeared that the splanchnic acceleration was the greater. This, of course, would be consistent with the view that the epinephrin secretion in response to direct splanchnic stimulation may play a substantial part in the reaction. It would appear probable that any share of the epinephrin in the acceleration both when the output is increased, as by splanchnic stimulation, and when, without an actual increase in the output, more epinephrin is sent through the coronary circulation in response to a rise of blood pressure caused by sensory nerve stimulation, must vary with the state of the heart and may, therefore, be expected to vary in different animals and in the same animal at different periods of an experiment.

Protocol. Cat 179; male; weight, 2.2 kgm. Under urethane (5 gm.) cut vago-sympathetics; excised stellate ganglia; prepared central end of left sciatic for stimulation.

		Rate	Pressure
4:00 p.m.	Before sciatic stimulation.....	169	80
	25 seconds after beginning stimulation.....	210	130
4:05 p.m.	Prepared peripheral end of left splanchnic (extraperitoneally)		
4:10 p.m.	Before stimulation of left splanchnic; left adrenal vein clipped.....	168	70
	20 seconds after beginning stimulation.....	182	94
4:13 p.m.	Before sciatic stimulation.....	173	81
	32 seconds after beginning stimulation.....	204	113
4:17 p.m.	Before stimulation of left splanchnic; left adrenal vein clipped.....	177	73
	20 seconds after beginning stimulation.....	180	98
4:20 p.m.	Before stimulation of left splanchnic.....	170	80
	24 seconds after beginning stimulation.....	183	109
4:25 p.m.	Before stimulation of sciatic; left adrenal vein clipped..	170	76
	23 seconds after beginning stimulation.....	180	106
4:28 p.m.	Before stimulation of left splanchnic; left adrenal vein clipped.....	170	71
	23 seconds after beginning stimulation.....	180	100
4:30 p.m.	Opened abdomen; manipulated intestines		
4:32 p.m.	Before sciatic stimulation.....	171	66
	25 seconds after beginning stimulation.....	196	102
4:35 p.m.	Before stimulation of sciatic; both adrenal veins clipped.....	173	64
	20 seconds after beginning stimulation.....	192	95
4:40 p.m.	Before stimulation of left splanchnic with both adrenal veins clipped.....	173	70
	21 seconds after beginning stimulation.....	179	103
4:45 p.m.	Before stimulation of splanchnic.....	171	62
	25 seconds after beginning stimulation.....	179	90
4:48 p.m.	Cut right splanchnic		
4:50 p.m.	Before sciatic stimulation.....	160	44
	23 seconds after beginning stimulation.....	161	56

The protocol of cat 179 shows again quite clearly good heart reactions with sciatic stimulation after opening the abdomen and section of one splanchnic. The practical disappearance of the reaction after section of the remaining splanchnic, when the blood pressure fell to 44 mm. of mercury is well shown. But is it likely that this disappearance is due solely to the suppression of the epinephrin secretion when a little earlier in the experiment a decided reaction was obtained by stimulation of the sciatic with both adrenal veins clipped and after section of the left splanchnic? Here the blood pressure reaction was a

good one, the pressure rising from 64 to 95 mm. of mercury. But after the section of the second splanchnic only a trifling rise of pressure was caused by sciatic stimulation. The absence of any substantial rise of pressure and the depressing influence of the lowered blood pressure on the paths for any other reflexes than the vascular reflexes which may be concerned in the reaction offer a more probable explanation.

In a number of cats one superior cervical ganglion (the left) had been previously excised, so that it was possible to compare the acceleration of the heart caused by stimulation of the sciatic or splanchnic with the eye reactions. The following is a typical protocol.

Protocol. Cat 190; female; weight, 2.31 kgm. Left superior cervical ganglion excised 21 days previously. Under urethane (5 gm.) cut vago-sympathetics; excised stellate ganglia; prepared central end of left sciatic for stimulation.

		Rate	Pressure
1:05 p.m.	Before stimulation of sciatic ²	253	123
	35 seconds after beginning stimulation.....	259	164
1:10 p.m.	Before sciatic stimulation ²	229	
	15 seconds after beginning stimulation.....	237	
1:15 p.m.	Prepared peripheral end of left splanchnic (extraperitoneally)		
1:35 p.m.	Before stimulation of left splanchnic.....	231	80
	10 seconds after beginning stimulation.....	268	155
	Very good pupil and nictitating reactions in 9 seconds		
1:40 p.m.	Before stimulation of left splanchnic with left adrenal vein clipped.....	223	64
	20 seconds after beginning stimulation.....	280	150
	Small pupil and nictitating reactions in 18.4 seconds		
1:50 p.m.	Before stimulation of left splanchnic with left adrenal vein clipped.....	227	70
	20 seconds after beginning stimulation.....	263	
	48 seconds after beginning stimulation.....	278	130
	Very small eye reactions in 24.8 seconds		
2:15 p.m.	Excised left adrenal		
2:20 p.m.	Before stimulation of left splanchnic.....	219	72
	25 seconds after beginning stimulation.....	227	100
	Doubtful, if any eye reactions		

² Both pupils dilate instantaneously, the left becoming much wider than the right, both returning to previous state very soon after stimulation of the sciatic is stopped. With a prolonged or strong stimulation the left nictitating may slowly retract. This reaction is distinctly different from that obtained with splanchnic stimulation (when the adrenal veins are free) which affects only the denervated eye and occurs after a distinct latent period.

2:30 p.m.	Before stimulation of sciatic.....	223	80
	22 seconds after beginning stimulation.....	262	168
2:35 p.m.	Opened abdomen; prepared peripheral end of right splanchnic		
2:40 p.m.	Before stimulation of right splanchnic.....	210	45
	20 seconds after beginning stimulation.....	285	105
2:55 p.m.	Before clipping aorta just above diaphragm.....	249	48
	After clipping aorta just above diaphragm.....	260	146

It is to be remarked that when the left splanchnic was stimulated with the left adrenal vein clipped, the eye reactions were not completely abolished but were much diminished and appeared after a much longer delay than when the splanchnic was stimulated with the vein open. Complete loss of the eye reactions on stimulating the peripheral end of a splanchnic nerve may be seen when the corresponding adrenal vein has been clipped off, although good reactions were being obtained with the vein open, or the result may be what was found in cat 190. This result is compatible with the view that some epinephrin may find its way into the blood stream by a collateral route, as suggested by Cow (12) (the renal vessels were not tied in this experiment*) but may also be due solely to a change in the concentration or quantity of epinephrin from the other adrenal passing through the coronary circulation, associated with the vasomotor effects of the splanchnic stimulation. The observation that in this experiment after excision of the left adrenal stimulation of the left splanchnic produced only a doubtful, if any, pupil reaction does not enable us to decide against the latter view. For the vasomotor effect (rise of blood pressure) and the heart acceleration were also much reduced, possibly owing to some injury to the splanchnic in removal of the adrenal. The fact that the eye reactions, while still present, were greatly diminished by the clipping of the adrenal vein and that it took a much greater time for them to appear while the acceleration of the heart was not at all diminished, shows clearly that the latter reaction cannot be a quantitative test for epinephrin. With sciatic stimulation the pupil reaction is complicated by the fact that both pupils dilate immediately after stimulation, of course through the nervous system. Although the sensitized pupil widens more than the other, this reaction is different from the typical epinephrin reaction yielded, for instance, by splanchnic stimulation. The return of the pupils to their previous size on stoppage of the stim-

*In making a cava pocket we generally tie the renal vein at the hilus and also at its entrance into the cava.

ulation begins at once and is accomplished more quickly after sciatic stimulation than when a true epinephrin reaction has been induced by splanchnic stimulation. Further, the pupil reaction elicited by stimulation of the sciatic after interference with the epinephrin output by removal of one adrenal and section of the nerves of the other, or after removal of both adrenals, has the same characters as that seen with intact adrenals. A nictitating reaction* was not obtained with sciatic stimulations which caused marked acceleration of the heart. Only with quite strong stimulation of the sciatic was there any retraction of the nictitating. This also is different from the genuine epinephrin eye reactions. The observations on the eye reactions, then, are inconsistent with the idea that stimulation of the sciatic causes a marked increase in the output of epinephrin, which in its turn causes the observed acceleration of the heart. It may further be asked how an acceleration (see protocol of cat 190) caused by sciatic stimulation, which is much smaller with both splanchnics intact at the beginning of the experiment than later on when one splanchnic has been divided and the corresponding adrenal removed, can be considered a quantitative reaction for the rate of output of epinephrin. It will be seen in another section of the paper that it is common, or indeed the rule, to obtain as large a reaction on stimulation of the sciatic in animals from which one adrenal has been removed and the animal allowed to recover as in animals with both adrenals intact.

Another cat (191) from which one superior cervical ganglion had been previously removed yielded results so similar to those of cat 190 that the protocol need not be reproduced. The only difference was that sciatic stimulation, as usual, gave a good acceleration at the beginning of the experiment, from 240 to a maximum of 295 beats per minute, the blood pressure rising from 124 to 182 mm. of mercury. With a second stimulation of the sciatic the heart rate increased from 256 to 275, the blood pressure rising from 112 to 150 mm. The eye reactions were the same as in cat 190. The abdomen was now opened and the peripheral end of the left splanchnic stimulated. The pulse rate rose from 243 to 270 beats, the blood pressure from 90 to 144 mm. of mercury. Excellent pupil and nictitating reactions were obtained in 9 seconds. The left adrenal vein was then clipped and the left splanchnic again stimulated. The heart rate increased from 237 to 262 beats per minute and the blood pressure from 110 to 150 mm. of mercury. Small pupil and nictitating reactions were observed in 20.4 seconds.

Protocol. Cat 195; female; weight, 2.4 kgm. Left superior cervical ganglion excised 14 days previously. Under urethane (5.5 gm.) prepared peripheral end of left splanchnic for stimulation in abdomen, tied lumbar veins just before they cross the adrenals; prepared central end of left sciatic for stimulation.

		Rate	Pressure
2:55 p.m.	Before stimulation of left splanchnic.....	202	78
	12 seconds after beginning stimulation.....	216	96
	Very good pupil and nictitating reactions in 12.8 seconds		
3:00 p.m.	Before stimulation of left splanchnic; left adrenal vein clipped.....	186	80
	19 seconds after beginning stimulation.....	211	108
	No eye reactions		
3:05 p.m.	Before stimulation of left splanchnic; left adrenal vein clipped.....	178	71
	14 seconds after beginning stimulation.....	181	78
3:20 p.m.	Cut vago-sympathetics and excised stellate ganglia		
3:30 p.m.	Before stimulation of sciatic ⁴	145	52
	20 seconds after beginning stimulation.....	169	94
3:35 p.m.	Before weaker stimulation of sciatic.....	152	63
	54 seconds after beginning stimulation.....	151	63
3:40 p.m.	Prepared peripheral end of right splanchnic in thorax		
3:42 p.m.	Before stimulation of right splanchnic.....	146	35
	40 seconds after beginning stimulation.....	192	76
	Good eye reactions in about 40 seconds		
3:45 p.m.	Before stimulation of right splanchnic; right adrenal vein clipped.....	157	44
	20 seconds after beginning stimulation.....	184	
	30 seconds after beginning stimulation.....	207	82
	No eye reactions		
3:50 p.m.	Before stimulation of right splanchnic; both adrenal veins clipped.....	150	44
	40 seconds after beginning stimulation.....	199	84
	No eye reactions		
3:55 p.m.	Before stimulation of right splanchnic.....	146	44
	30 seconds after beginning stimulation.....	200	68
	Good eye reactions occurred in 30-35 seconds		

In the above experiment (cat 195) the abdomen was opened, the lumbar vein tied just before it crosses the left adrenal and the left splanchnic stimulated several times before the vago-sympathetics were cut and the stellate ganglia excised. The central end of the sciatic was then stimulated and caused an acceleration of 24 beats and a rise of blood pressure from 52 to 94 mm. of mercury, showing again that Cannon's statement that the heart reaction is scarcely ever obtained with sciatic stimulation after opening the abdomen is unfounded.

⁴ Eye reactions with sciatic stimulation as described in protocol of cat 190, p. 319.

Of course when a weak enough stimulus was employed (5 minutes thereafter) the heart rate remained unchanged, but so did the blood pressure. Precisely the same result was obtained with stimulation of the peripheral end of the splanchnic, a stimulus which failed to cause any appreciable rise of blood pressure (as at 3:05 p.m.) also caused little if any acceleration of the heart. Whether the corresponding adrenal vein was open or clipped seemed to have no influence on the maximum acceleration. Indeed in the first splanchnic stimulation (at 2:55 p.m.) with the vein open the maximum acceleration was only 14 beats, whereas in the next stimulation, with the vein clipped, it was 25 beats per minute. Yet the eye reactions, which were very good in the first case, were abolished in the second. The eye reactions are universally admitted to be due to epinephrin, when elicited by stimulation of the peripheral end of the splanchnic. How is it possible to believe that when they are negative, while the heart acceleration is even greater than when they were strongly positive, the acceleration of the heart is a specific reaction for epinephrin?

In another cat (196), in which the left superior cervical ganglion had been excised 20 days previously, the vago-sympathetics were cut, the stellate ganglia excised and the abdomen opened under urethane at the beginning of the experiment. The lumbar veins, just before crossing the adrenals, and the renal arteries and veins were tied on both sides and both adrenal veins prepared for clipping. The peripheral end of the left sympathetic in the thorax was prepared for stimulation and the central end of a sciatic. Stimulation of the left sympathetic with the adrenal veins open gave a good pupil reaction in 8 seconds. The heart rate, which was 212 before stimulation, reached a maximum of 255 beats per minute (counting from 16 seconds after beginning of stimulation). The blood pressure rose from 72 to 150 mm. of mercury. The left sympathetic was now stimulated with the left adrenal vein clipped. There was no pupil reaction. The heart rate increased from 201 to a maximum of 260 beats per minute (counting from 16 seconds after the beginning of stimulation) and the blood pressure from 94 to 134 mm. of mercury. Then the left sympathetic was again stimulated with both adrenal veins clipped. There was no pupil reaction but the heart rate increased from 200 to a maximum of 245 beats a minute and the blood pressure from 58 to 107 mm. of mercury. Is not this again quite inconsistent with the view that the heart acceleration constitutes a quantitative reaction by which the output of epinephrin can be determined?

The next protocol (from cat 200) illustrates the general parallelism between the acceleration of the heart elicited by sciatic stimulation and the rise of blood pressure, and the absence of any demonstrable influence of clipping of the adrenal veins on either the maximum acceleration or the increase of blood pressure.

Protocol. Cat 200; female; weight, 1.47 kgm. Left superior cervical ganglion excised 16 days previously. Under urethane anesthesia cut vago-sympathetics; excised stellate ganglia; prepared central end of sciatic for stimulation.

		Rate	Pressure
10:00 a.m.	Before sciatic stimulation ⁵	210	84
	Counts of successive portions of the curve after beginning of stimulation (with progressive increase in strength).....	211	84
		215	102
		230	112
		230	128
		244	128
10:05 a.m.	Before sciatic stimulation.....	214	100
	Counts of successive portions of the curve with stimulation as above.....	224	117
		240	126
		240	118
10:10 a.m.	Prepared adrenal veins for clipping (extraperitoneally)		
10:15 a.m.	Before sciatic stimulation.....	217	84
	Successive counts after beginning of stimulation as above.....	223	100
		234	110
		248	110
10:20 a.m.	Before sciatic stimulation; both adrenal veins clipped.	206	70
	Successive counts after beginning of stimulation as above.....	212	88
		222	89
		223	88
		225	86
	Just after release of adrenal veins.....	228	95
	15 seconds after release of adrenal veins.....	245	104
10:25 a.m.	Before sciatic stimulation.....	214	80
	Successive counts as above during stimulation.....	216	88
		239	104
		253	117
10:30 a.m.	Before sciatic stimulation; both adrenal veins clipped.	215	77
	During stimulation as above.....	227	84
		228	98
	Just after release of adrenal veins.....	236	96
	12 seconds after release of adrenal veins.....	239	100

In the last experiment (cat 193) to be quoted in this section of the paper, only the vago-sympathetics were cut at the beginning of the experiment, and the abdomen was opened.

⁵ The effects on the eye were those described in the footnote to protocol of cat 190, p. 319.

Protocol. Cat 193; male; weight, 2.7 kgm. Left superior cervical ganglion excised 10 days previously. Under urethane (5 gm.) opened abdomen; prepared peripheral end of left splanchnic for stimulation; cut vago-sympathetics.

		Rate	Pressure
12:08 p.m.	Before stimulation of splanchnic; left adrenal vein clipped.....	157	98
	11 seconds after beginning stimulation.....	175	
	18 seconds after beginning stimulation.....	180	145
	No eye reactions during stimulation; after release of clip good pupil and nictitating reactions in 9.2 seconds		
12:10 p.m.	Before stimulation of splanchnic.....	162	96
	10 seconds after beginning stimulation.....	209	136
	Good pupil and nictitating reactions in 11.5 seconds		
12:15 p.m.	Before stimulation of splanchnic; left adrenal vein clipped.....	172	120
	17 seconds after beginning stimulation.....	190	162
	No eye reactions during stimulation; on release of clip good pupil and nictitating reactions in 8.2 seconds		
12:20 p.m.	Excised stellate ganglia		
12:40 p.m.	Before stimulation of splanchnic; left adrenal vein clipped.....	185	114
	18 seconds after beginning stimulation.....	201	144
	No eye reactions during stimulation; on release of clip good pupil and nictitating reactions in 11 seconds		
12:50 p.m.	Stimulated splanchnic with left adrenal vein and coeliac and superior mesenteric arteries clipped.		
	Before stimulation.....	203	134
	17 seconds after beginning stimulation.....	202	136
	No eye reactions during stimulation; on release of adrenal vein good pupil and nictitating reactions in 10.6 seconds		
	Before removal of adrenal clip.....	202	121
	12 seconds after release of adrenal vein.....	238	128
	Removed clips from coeliac and superior mesenteric		
1:00 p.m.	Prepared central end of sciatic for stimulation		
1:02 p.m.	Before stimulation of sciatic.....	174	78
	11 seconds after beginning stimulation.....	184	102
	20 seconds after beginning stimulation.....	194	
	34 seconds after beginning stimulation.....	200	104
1:20 p.m.	Before stimulation of sciatic.....	184	68
	16 seconds after beginning stimulation.....	200	108

The effects of stimulating the splanchnic, with the corresponding adrenal vein open and clipped, were not obviously different from those in the other experiments, where the stellate ganglia had been excised at the beginning, nor did removal of the stellate ganglia cause any essential change. The failure of the heart reaction with splanchnic

stimulation when the coeliac and superior mesenteric arteries were clipped is clearly associated with the absence of a rise of pressure when the splanchnic area is thus eliminated. On removal of the clip from the adrenal vein the usual good eye reactions were obtained and also an acceleration of the heart due to release of the epinephrin pent up in the adrenal vessels.

It may be tedious to point out again that an hour and a half after the abdomen had been opened, with one splanchnic cut, after all the clipping and unclipping of the adrenal veins and of the coeliac and superior mesenteric arteries (and a good many observations actually made have been omitted from the protocol to save space) stimulation of the sciatic still gave a good acceleration of the heart, although, according to Cannon, no reaction ought to have been obtained. In view of such facts what becomes of Doctor Cannon's assertion, unsupported by any evidence that when we collect adrenal vein blood from a cava pocket, the "peculiar" conditions of our experiments do not permit of the demonstration that with stimulation of sensory nerves there is a vast outpouring of epinephrin from the adrenals, a demonstration which he obtains by a misinterpretation of a heart reaction equally well elicited whether the adrenal veins are open or clipped off, whether the eye (sensitized by removal of the superior cervical ganglion) is giving negative or positive reactions for epinephrin? Since, however, Doctor Cannon maintains that the mere clipping or tying of the adrenal veins need not interfere in the least with the passage of epinephrin to the heart, and has even convinced himself that adrenalin, injected into the lumbar vein crossing the adrenal after it has been ligated on each side of the gland, passes so freely into the circulation that it causes a large rise of blood pressure, evidence of another kind will now be given that the acceleration of the heart relied upon by him to prove a markedly augmented rate of epinephrin output when the sciatic nerve is stimulated has no such significance.

EXPERIMENTS ON ANIMALS WITH ONE ADRENAL REMOVED AND THE NERVES OF THE OTHER CUT

We have previously shown (13) that after this operation the epinephrin output is either greatly reduced or abolished, within the limits of sensitiveness of the test objects (rabbit's intestine and uterus segments) employed to detect and estimate it. It was therefore of interest to see whether the acceleration of the heart would

be obtained in these animals by stimulation of sensory nerves. The result was positive in all the cats, a good acceleration being elicited by stimulation of the sciatic. For example, in cat 201, a female weighing 2.575 kgm., the right adrenal was removed, the nerves of the left including the splanchnic cut. A portion of the left semilunar ganglion was removed. The left superior cervical ganglion was excised at the same time. Eighteen days thereafter, the animal being in good condition, the vago-sympathetics were cut and the stellate ganglia removed under urethane and the central end of the left sciatic nerve prepared for stimulation. A blood pressure tracing was taken as usual from the right carotid. Stimulation of the sciatic caused the heart rate to increase from 177 before stimulation to a maximum rate of 211 beats per minute. The blood pressure increased from 54 mm. before stimu-

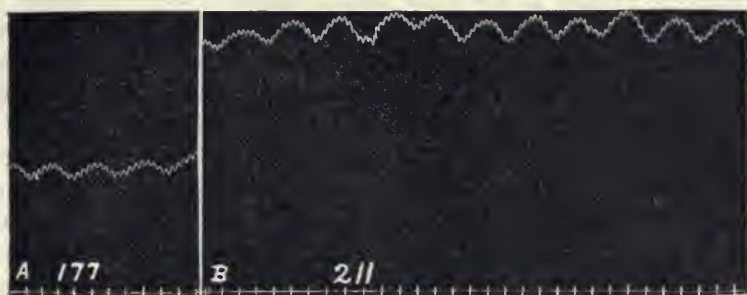


Fig. 4. Parts of blood pressure tracings from cat 201. A, before and B, a portion commencing 20 seconds after beginning of sciatic stimulation. Reduced to three-fifths.

lation to a maximum of 132 mm. of mercury. The acceleration and the blood pressure increased together. Successive portions of the curve starting from the point of stimulation yielded the following heart rates and blood pressures 180 (72), 193 (97), 202 (107), 209 (118), 209 (132). The blood pressures are in parentheses. A sample of the curve is reproduced in figure 4. The effect of sciatic stimulation on the pupils did not differ materially from that already described in cats in which the epinephrin output had not been interfered with (see footnote to protocol of cat 190). The nictitating membrane did not move. The abdomen was afterwards opened, a short cava pocket made and adrenal vein blood collected, which was assayed on rabbit segments. It was shown that the epinephrin output could not have been more than one-fiftieth of the average output per kilogram per minute, under

the conditions of our experiments. The preliminary operation had, therefore, effectively severed the epinephrin-secretory nerves of the left adrenal. In spite of this and also in spite of the fact that one splanchnic had been divided, and with both splanchnics intact a greater reflex rise of blood pressure might have been obtained, a maximum acceleration of the heart of 32 to 34 beats was elicited by stimulation of the sciatic. According to Cannon, the whole of the acceleration must have been due to a great outpouring of epinephrin from the left adrenal, reflexly stimulated along efferent nerve paths which had been divided 18 days before, and which certainly had not regenerated in that time.

In another cat (202), a female weighing 2.545 kgm., the right adrenal was removed and the nerves of the left cut. The left superior cervical ganglion was excised at the same time. Eighteen days thereafter the heart was denervated under urethane and blood pressure tracings taken from the right carotid. Stimulation of the central end of the sciatic caused an acceleration of the heart rate from 145 per minute before stimulation to 161. The blood pressure rose from 92 to 134 mm. of mercury. Successive portions of the curve, from the beginning of stimulation, yielded the following heart rates and blood pressures: 152 (100), 155 (110), 157 (130), 160 (126), 161 (134). The pupil reactions were as previously described in the footnote to the protocol of cat 190. There was no movement of the nictitating membrane. After the sciatic stimulations the abdomen was opened, and 3 specimens of adrenal vein blood collected—the first, about 1 gram (discarded), the second, 2.9 grams in 4 minutes (0.75 gm. per minute), the third 5.1 grams in 9 minutes (0.57 gm. per minute). No evidence was obtained with rabbit segments that the blood contained any epinephrin. It was shown that the third specimen could not have contained 1:70,000,000 epinephrin, i.e., the output could not have been 0.000007 mgm. per minute for the cat, or 0.0000027 mgm. per kilogram per minute. In other words it could not have been one-hundredth of the average normal output, and there was no evidence that any epinephrin was being given off. The interval after the preliminary operation was quite short in this animal, 8 days, which possibly may be an unfavorable condition for obtaining a large heart reaction, although we have no evidence as to this.

In the next experiment (cat 215) the animal was allowed to survive for 2 months.

Protocol. Cat 215; female; weight 2.38 kgm.

May 17, 1918. Right adrenal excised, left denervated and right superior cervical ganglion excised.

July 15, 1918. Under urethane (5 gm.) cut vago-sympathetics, excised stellate ganglia, prepared central end of left sciatic for stimulation.

		Rate	Pressure
11:15 a.m.	Before sciatic stimulation for 38 seconds (8 cm.) ⁶	219	134
	During first 14 seconds of stimulation.....	232	162
	During next 13 seconds of stimulation.....	238	170
	During next 13 seconds.....	244	172
	During next 20 seconds.....	228	147
11:20 a.m.	Before sciatic stimulation for 33 seconds (6 cm.).....	222	144
	During first 14 seconds of stimulation.....	240	180
	During next 14 seconds of stimulation.....	248	196
	During next 15 seconds.....	249	180
11:25 a.m.	Before sciatic stimulation for 26 seconds (6.5 cm.).....	219	152
	During first 8 seconds of stimulation.....	226	178
	During next 7 seconds of stimulation.....	230	182
	During next 12 seconds.....	246	190
11:30 a.m.	Before sciatic stimulation for 40 seconds (6 cm.).....	224	148
	During first 13 seconds of stimulation.....	239	177
	During next 14 seconds of stimulation.....	252	180
	During next 13 seconds of stimulation.....	251	182
	During next 10 seconds.....	248	168
11:35 a.m.	Before sciatic stimulation for 37 seconds (4 cm.).....	222	148
	During first 12 seconds of stimulation.....	239	173
	During next 11 seconds of stimulation.....	244	180
	During next 11 seconds of stimulation.....	252	180
	During next 14 seconds.....	249	168

Later on the abdomen was opened and adrenal vein blood collected. Assays of the adrenal blood released from a cava pocket were also made by the pupil reaction.

The protocol shows that stimulation of the sciatic caused good acceleration of the heart with corresponding changes in the blood pressure. The rabbit intestine assay of the adrenal blood demonstrated that a substantial output of epinephrin was still going on. The concentration of the second specimen was about 1:6,500,000 and that of the third specimen about 1:3,750,000, corresponding in either case to an output of 0.00008 mgm. per minute for the cat, or 0.00003 mgm. per kilogram per minute, one-seventh or one-eighth of the average normal output. It is impossible to say whether an unusually large proportion of the secretory fibers of the left adrenal had escaped

⁶ Eye reactions, as described in footnote to protocol of cat 190, p. 319. The distance between the coils is given in brackets.

section at the preliminary operation, or whether some regeneration had occurred. In any case the innervation of the one adrenal remaining must still have been seriously crippled. Yet the heart reaction on which Cannon relies as an "indicator of adrenal secretion" was as well obtained with sciatic stimulation as in normal animals, corresponding with the general excellent condition of the cat two months after the primary operation.

In two cats operated on in the same way but only a relatively short time before (8 days and 7 days), the experiment was completed by excising the remaining (already denervated) adrenal. It was not thought advisable in these cats to complicate the experiment by attempting to estimate the residual epinephrin output, if any, on the adrenal vein blood. But it was shown that the epinephrin store of the adrenal constituted a full load, a good indication that protection of the gland from depletion during the experiment was relatively complete.

Protocol. Cat 444; male; weight, 2.9 kgm. Right adrenal excised and left denervated 8 days, left superior cervical ganglion excised 14 days previously. Under ether cut vago-sympathetics, excised stellate ganglia, prepared central end of right sciatic for stimulation.

		Rate	Pressure
10:40 a.m.	Before sciatic stimulation (8 cm.)	133	132
	2 seconds after beginning stimulation	130	128
	30 seconds after beginning stimulation	132	130
10:45 a.m.	Before sciatic stimulation (5 cm.)	133	112
	6 seconds after beginning stimulation	140	
	40 seconds after beginning stimulation	152	92
	60 seconds after beginning stimulation	145	106
10:50 a.m.	Before sciatic stimulation (8 cm.)	127	108
	3 seconds after beginning stimulation	132	
	30 seconds after beginning stimulation	136	100
	45 seconds after beginning stimulation	131	104
10:55 a.m.	Before sciatic stimulation (6 cm.)	121	120
	5 seconds after beginning stimulation	130	126
11:10 a.m.	Excised left adrenal (extraperitoneally)		
11:15 a.m.	Before sciatic stimulation (8 cm.)	130	103
	3 seconds after beginning stimulation	133	96
	23 seconds after beginning stimulation	140	
	60 seconds after beginning stimulation	144	114
11:48 a.m.	Opened abdomen, tied coeliac axis and superior mesenteric artery		
11:50 a.m.	Before sciatic stimulation (6 cm.)	139	74
	8 seconds after beginning stimulation	144	74
	3 seconds after stopping stimulation	142	72
	40 seconds after stopping stimulation	134	70

11:58 a.m.	Before sciatic stimulation (4 cm.).....	123	72
	2 seconds after beginning stimulation.....	130	77
	15 seconds after beginning stimulation.....	133	80
	40 seconds after beginning stimulation.....	137	81
	60 seconds after beginning stimulation.....	138	78
12:05 p.m.	Before sciatic stimulation (8 cm.).....	130	76
	2 seconds after beginning stimulation.....	133	80
	30 seconds after beginning stimulation.....	135	82

In cat 444 the greatest acceleration caused by sciatic stimulation before excision of the remaining adrenal was 19 beats. With repeated stimulations the acceleration effect declined. The last stimulation before excision gave only 9 beats. A weaker stimulation just after excision gave a maximum acceleration of 14 beats, and another stronger stimulation gave a maximum acceleration of 15 beats. Even after the abdomen was opened and the superior mesenteric artery and coeliac axis tied, a maximum acceleration of 15 beats was obtained in the absence of the adrenals.

In cat 441, also, while the greatest acceleration before excision of the remaining adrenal (17 beats) was not reached afterwards, the acceleration immediately after excision was the same as that obtained just before it (9 beats), although the stimulation in the latter case was the stronger.

Protocol. Cat 441; female; weight, 1.66 kgm. Right adrenal excised and left denervated 7 days previously. Under ether cut vago-sympathetics, excised stellate ganglia, prepared central end of right sciatic for stimulation.

		Rate	Pressure
10:55 a.m.	Before sciatic stimulation (10 cm.).....	219	100
	6 seconds after beginning stimulation.....	229	116
	20 seconds after beginning stimulation.....	228	
10:58 a.m.	Before sciatic stimulation (8 cm.).....	217	115
	6 seconds after beginning stimulation.....	223	122
11:02 a.m.	Before sciatic stimulation (6 cm.).....	208	106
	10 seconds after beginning stimulation.....	215	118
	30 seconds after beginning stimulation.....	213	
11:06 a.m.	Before sciatic stimulation (6 cm.).....	211	109
	5 seconds after beginning stimulation.....	217	
	18 seconds after beginning stimulation.....	228	120
	28 seconds after beginning stimulation.....	227	
11:10 a.m.	Before sciatic stimulation (10 cm.).....	207	113
	5 seconds after beginning stimulation.....	215	116
11:15 a.m.	Before sciatic stimulation (8 cm.).....	206	109
	5 seconds after beginning stimulation.....	222	139

		Rate	Pressure
11:20 a.m.	Before sciatic stimulation (7 cm.).....	216	116
	5 seconds after beginning stimulation.....	224	148
11:25 a.m.	Before sciatic stimulation (6 cm.).....	212	98
	5 seconds after beginning stimulation.....	221	119
11:40 a.m.	Excised left adrenal (lumbar route)		
11:42 a.m.	Before sciatic stimulation (8 cm.).....	214	86
	5 seconds after beginning stimulation.....	220	
	20 seconds after beginning stimulation.....	223	114
11:45 a.m.	Before sciatic stimulation (10 cm.).....	211	84
	5 seconds after beginning stimulation.....	214	100
	23 seconds after beginning stimulation.....	215	
11:51 a.m.	Before sciatic stimulation (9 cm.).....	210	70
	6 seconds after beginning stimulation.....	214	88
11:55 a.m.	Before sciatic stimulation (7 cm.).....	208	70
	6 seconds after beginning stimulation.....	210	84
11:58 a.m.	Before sciatic stimulation (8 cm.).....	211	68
	16 seconds after beginning stimulation.....	218	97
12:00 m.	Prepared central end of left sciatic for stimulation		
12:05 p.m.	Before stimulation of left sciatic (9 cm.).....	214	68
	30 seconds after beginning stimulation.....	217	72
12:10 p.m.	Before stimulation of right sciatic (10 cm.).....	215	68
	5 seconds after beginning stimulation.....	216	76
12:15 p.m.	Before stimulation of right sciatic (8 cm.).....	236	55
	5 seconds after beginning stimulation.....	242	62
	11 seconds after beginning stimulation.....	245	

The left adrenal weighed 0.226 gm. and contained 0.23 mgm. of epinephrin at end of experiment.

In the last cat (450) of this series to be mentioned a longer period (34 days) was allowed to elapse between the primary operation and the experiment, in order that the animal might have more fully recovered.

Protocol. Cat 450; male; weight, 2.96 kgm. Right adrenal excised and left denervated (with section of left splanchnic as usual) 34 days previously. Under urethane (5 gm.) cut vago-sympathetics; excised stellate ganglia; prepared central end of left sciatic for stimulation.

		Rate	Pressure
11:10 a.m.	Before sciatic stimulation (10 cm.).....	173	102
	During first 18 seconds of stimulation.....	178	114
	During next 20 seconds of stimulation.....	182	114
	Just after end of stimulation.....	185	
	30 seconds after end of stimulation.....	180	
11:14 a.m.	Before sciatic stimulation (9 cm.).....	174	100
	During first 12 seconds of stimulation.....	178	
	During next 16 seconds of stimulation.....	183	133
	Just after end of stimulation.....	191	126
	20 seconds after end of stimulation.....	189	129

		<i>Rate</i>	<i>Pressure</i>
11:18 a.m.	Before sciatic stimulation (8 cm.).....	171	97
	During first 16 seconds of stimulation.....	178	
	During next 17 seconds of stimulation.....	187	134
	Just after end of stimulation.....	192	134
	20 seconds after end of stimulation.....	190	
11:40 a.m.	Excised left adrenal (extraperitoneally)		
11:43 a.m.	Before sciatic stimulation (9 cm.).....	174	94
	During first 15 seconds of stimulation.....	179	130
	During next 20 seconds of stimulation.....	189	127
11:48 a.m.	Before sciatic stimulation (8 cm.).....	174	103
	During first 14 seconds of stimulation.....	178	132
	During next 20 seconds of stimulation.....	191	144
	Just after end of stimulation.....	200	150
	15 seconds after end of stimulation.....	198	128
	35 seconds after end of stimulation.....	194	127
11:58 a.m.	Before sciatic stimulation (7 cm.).....	177	96
	During first 17 seconds of stimulation.....	181	128
	During next 20 seconds of stimulation.....	192	138
	Just after end of stimulation.....	200	122
	20 seconds after end of stimulation.....	195	120
12:10 p.m.	Prepared peripheral end of right splanchnic (in thorax)		
12:12 p.m.	Before splanchnic stimulation (10 cm.).....	167	70
	During first 20 seconds of stimulation.....	176	96
	During next 20 seconds of stimulation.....	184	104
	Just after end of stimulation.....	186	92
12:15 p.m.	Before splanchnic stimulation (8 cm.).....	177	61
	During first 20 seconds of stimulation.....	175	70
	During next 20 seconds of stimulation.....	185	90
	Just after end of stimulation.....	189	86
12:21 p.m.	Before splanchnic stimulation (7 cm.).....	178	60
	During first 16 seconds of stimulation.....	179	93
	During next 20 seconds of stimulation.....	186	108
	Just after end of stimulation.....	187	96
12:26 p.m.	Before sciatic stimulation (7 cm.).....	179	62
	During first 20 seconds of stimulation.....	182	83
	During next 20 seconds of stimulation.....	180	72
12:35 p.m.	Cut left splanchnic in thorax		
12:36 p.m.	Before sciatic stimulation (7 cm.).....	181	63
	During first 18 seconds of stimulation.....	182	82
	During next 20 seconds of stimulation.....	181	78
	Just after end of stimulation.....	184	74
1:01 p.m.	Before splanchnic stimulation (7 cm.).....	183	46
	During first 16 seconds of stimulation.....	184	78
	During next 20 seconds of stimulation.....	196	88
	Just after end of stimulation.....	208	74
	15 seconds after end of stimulation.....	209	65
	22 seconds after end of stimulation.....	212	60

It will be seen from the protocol that a good acceleration was obtained on sciatic stimulation before removal of the remaining (already denervated) adrenal, (12 beats, 17 beats, 21 beats per minute in successive observations with different strength of stimulation). After removal of the adrenal the accelerations obtained were fully as great as before (15 beats, 26 beats, 23 beats per minute). When, however, in the absence of the adrenals, the right splanchnic was cut in the thorax sciatic stimulation caused practically no acceleration, and the rise of pressure was, of course, much reduced (fig. 5). This was not due to any change in the condition of the animal which rendered direct stimulation of the splanchnic less effective, for the rise of pressure and the acceleration on stimulating the peripheral end of the right splanchnic

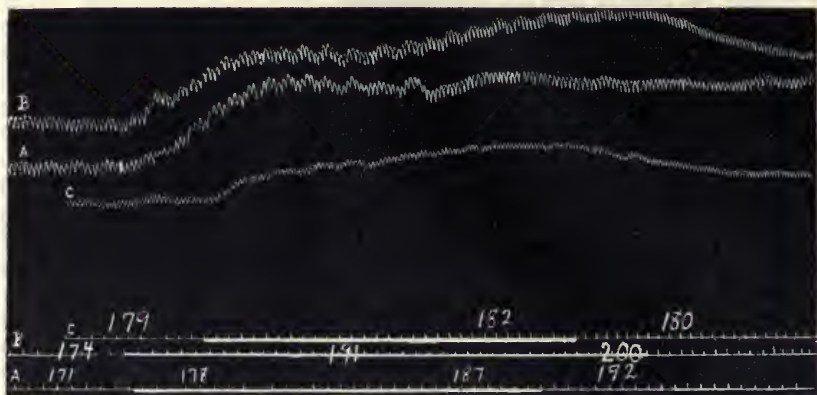


Fig. 5. Blood pressure curves from cat 450. A, sciatic stimulation before and B, after excision of remaining (already denervated) adrenal; C, after section of remaining splanchnic. Reduced to three-fifths.

was quite as great as before the last sciatic stimulation (as much as 29 beats per minute), (fig. 6). The failure of the heart reaction with sciatic stimulation after section of the right splanchnic (the left had been divided at the primary operation) is precisely what Cannon describes as occurring after removal of the adrenals. But in this animal the removal of the remaining adrenal did not affect the heart reaction, while subsequent section of the remaining splanchnic abolished it. This is incompatible with Cannon's interpretation of the failure of the reaction after section of the splanchnics as due entirely to interference with the epinephrin output. In the present experiment, to be logical, he would have to attribute the result to the loss of something coming from the liver (sugar?) or from the intestines, mobilized reflexly through the splanchnic.

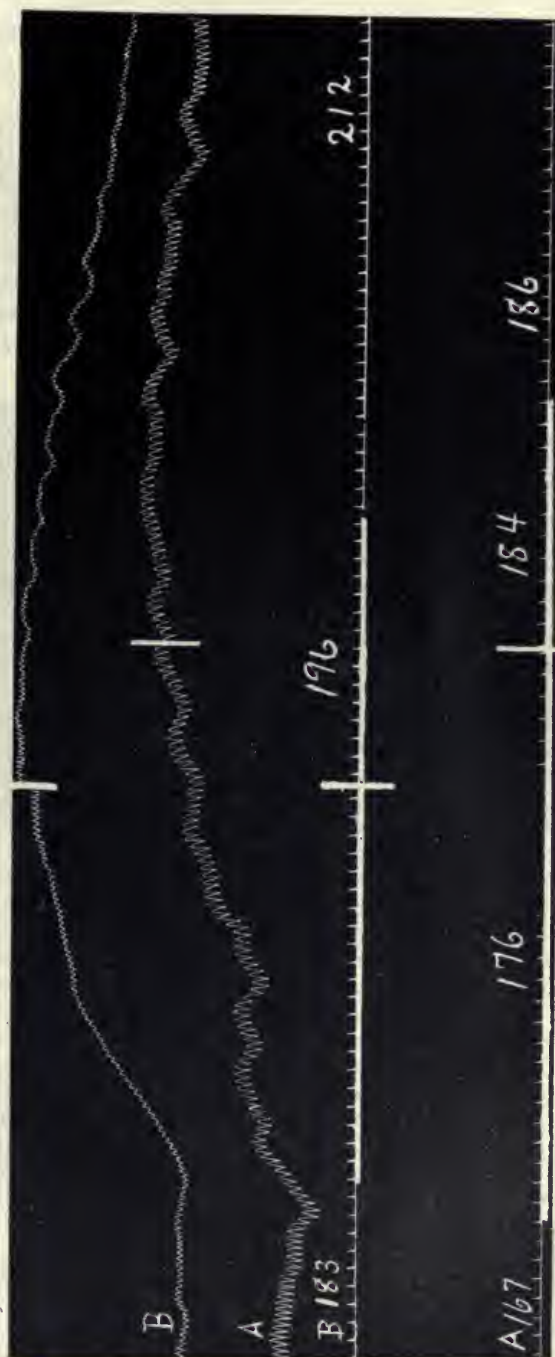


Fig. 6. Blood pressure curves from cat 450. A, splanchnic stimulation [after excision of remaining (already denervated) adrenal]; B, same, with stronger stimulus, 50 minutes later (7 seconds of each trace cut out to save space).

EXPERIMENTS WITH AN INTERVAL BETWEEN REMOVAL OF THE TWO ADRENALS

To avoid division of one splanchnic and to make the first operation less severe than the ordinary operation for suppression of the epinephrin output, while making the second operation less severe than the removal of both adrenals at one time, a number of experiments were performed in which one adrenal was removed, and then, after an interval of 3 to 13 days, the observations on sciatic stimulation with denervation of the heart and excision of the remaining adrenal were made. In cat 440, for example, the experiment was performed 6 days after the removal of the right adrenal.

Protocol. Cat 440; male; weight, 2.31 kgm. Right adrenal excised 6 days previously. Under ether cut vago-sympathetics, excised stellate ganglia, prepared central end of right sciatic for stimulation.

		Rate	Pressure
10:45 a.m.	Before sciatic stimulation (10 cm.).....	226	134
	5 seconds after beginning stimulation.....	256	168
10:49 a.m.	Before sciatic stimulation (8 cm.).....	220	128
	6 seconds after beginning stimulation.....	253	171
	15 seconds after end of stimulation.....	264	156
11:00 a.m.	Excised left adrenal (extraperitoneally)		
11:02 a.m.	Before sciatic stimulation (8 cm.).....	212	124
	5 seconds after beginning stimulation.....	242	
	15 seconds after beginning stimulation.....	255	156
	23 seconds after beginning stimulation.....	250	156
	37 seconds after beginning stimulation.....	228	138
	60 seconds after beginning stimulation.....	218	134
11:05 a.m.	Before sciatic stimulation (6 cm.).....	213	119
	6 seconds after beginning stimulation.....	252	154
	28 seconds after beginning stimulation.....	247	
11:09 a.m.	Before sciatic stimulation (4 cm.).....	212	118
	5 seconds after beginning stimulation.....	230	133
	22 seconds after beginning stimulation.....	248	153
11:20 a.m.	Before sciatic stimulation (8 cm.).....	221	112
	6 seconds after beginning stimulation.....	236	130
	22 seconds after beginning stimulation.....	247	140
11:25 a.m.	Before sciatic stimulation (6 cm.).....	230	118
	8 seconds after beginning stimulation.....	241	130
11:28 a.m.	Before sciatic stimulation (4 cm.).....	225	133
	7 seconds after beginning stimulation.....	231	146
	43 seconds after beginning stimulation.....	227	130
11:45 a.m.	Exposed cord in midcervical region		
11:50 a.m.	208	90

		Rate	Pressure
11:55 a.m.	Before total asphyxia (for 45 seconds).....	207	84
	3 seconds after beginning asphyxia.....	204	
	10 seconds after beginning asphyxia.....	225	88
	55 seconds after beginning asphyxia.....	210	88
12:05 a.m.	3 minutes after transection of cord between 4th and 5th cervical segments.....	193	51

As will be seen from the protocol, excellent heart reactions were obtained both before and after the removal of the remaining adrenal in cat 440. It is to be particularly noted that excision of the left adrenal left the blood pressure and heart rate practically unchanged.

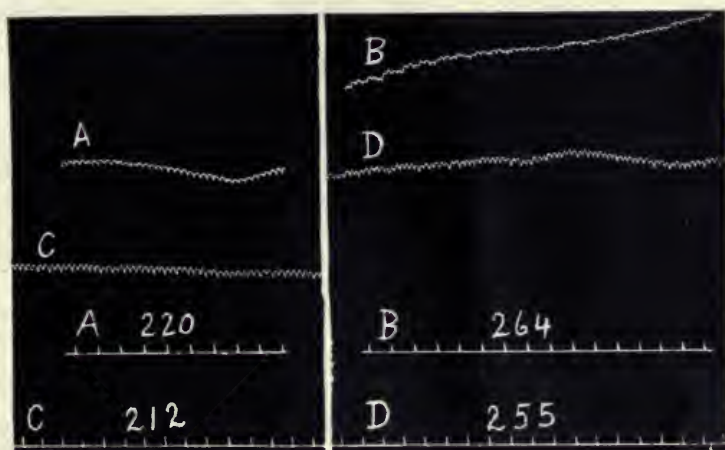


Fig. 7. Parts of blood pressure tracings from cat 440. *A*, before and *B*, a portion commencing 15 seconds after beginning of sciatic stimulation, before excision of remaining adrenal; *C*, before and *D*, 15 seconds after beginning of sciatic stimulation, after excision of remaining adrenal. Zero line moved up 41 mm.

The maximum acceleration for the last sciatic stimulation prior to removal of the left adrenal was 33 beats per minute, and for the first sciatic stimulation after removal of the adrenal 42 beats, the maximum pulse rate reached being practically identical in the two cases (253, 250). Samples of the curves used in counting the pulse rate are given in figure 7. The greater portion of each of the two curves, much reduced, is reproduced in figure 8, to show that excision of the second adrenal has not in any way essentially changed the vascular reaction. Here was a cat, then, without adrenals in which the acceleration produced by stimulation of the sciatic was actually greater than that

produced by a similar stimulation while one adrenal was still intact. Yet, according to Cannon, the last acceleration, like the first, must have been due solely to augmented epinephrin output from the adrenals. It should be noted that the exposure of the cord in the midcervical region led to a drop of blood pressure to 90 mm. of mercury and a corresponding slowing of the heart to 208 beats a minute. Subsequent transection of the cord caused the blood pressure to fall to 51 mm. of mercury and the heart rate to 193. It must be remembered that the adrenals were out, and the decrease in the heart rate can have nothing to do with absence of adrenal epinephrin. Cannon has emphasized the fact that after removal of the adrenals the pulse rate drops. Our observations show that this is always true provided the blood pressure

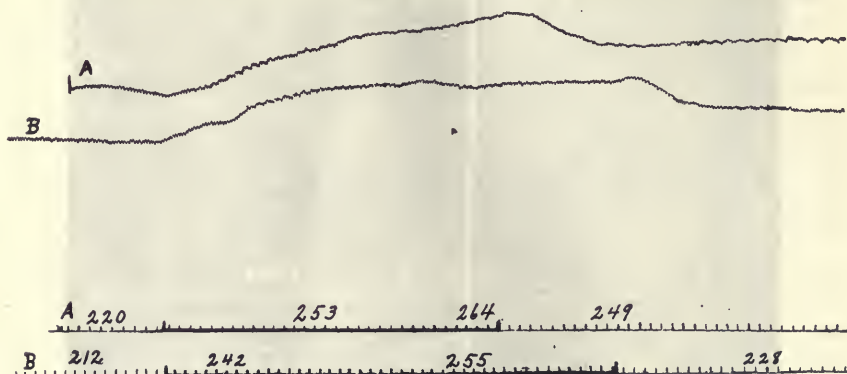


Fig. 8. Blood pressure curves from cat 440. A, sciatic stimulation before and B, after removal of remaining adrenal. Reduced to one-half.

falls decidedly. But if the pressure is maintained there is not necessarily any sensible slowing of the heart. The diminution in the pulse rate is, therefore, no index of the previous rate of output of epinephrin. This statement of observed facts is made without prejudice to the question whether the epinephrin liberated at the ordinary rate, under our experimental conditions, is capable of exerting an influence upon the heart, including its rate. We have brought forward some evidence that there is such an influence. But it may not be obvious after suppression of the epinephrin output when the blood pressure is well maintained. In cat 440 repeated stimulation of the sciatic caused some exhaustion of the heart reaction after removal of the last adrenal. But this, of course, is true of all reflexes, and in the case of the heart reaction can occur also with intact adrenals.

In the next experiment (cat 443) an example is given in which the heart acceleration elicited by sciatic stimulation was small from the beginning, but quite as good reactions were obtained after removal of the second adrenal as before (greatest acceleration before removal of the adrenal, 7 beats in one observation and 9 beats in another; after removal, 10 beats in one observation and 11 beats in another). It will be noted that as the blood pressure continued to fall progressively the heart rate diminished also, and this was not related to the adrenalectomy. Thus half an hour after removal of the adrenal, the pulse rate was 160 and the blood pressure 76. When the blood pressure had fallen to 50 the heart rate was 147. Before the removal of the adrenal the pulse rate was 180, 179 and 200 in 3 observations. After the adrenalectomy it was 182, the blood pressure being 83 mm. of mercury instead of 110 mm. at the last observation. As in other protocols counts of the heart rate at different parts of the curve and the results of stimulation of different strengths are given in order to show that the maximum accelerations quoted were really the maximum obtainable, under our conditions, in each animal.

Protocol. Cat 443; female; weight, 1.53 kgm. Right adrenal excised 7 days, left superior cervical ganglion excised 13 days previously. Under ether cut vago-sympathetics, excised stellate ganglia, prepared central end of left sciatic for stimulation.

		Rate	Pressure
10:55 a.m.	Before sciatic stimulation (10 cm.) ⁷	180	116
	6 seconds after beginning stimulation.....	187	148
10:59 a.m.	Before sciatic stimulation (9 cm.).....	179	106
	6 seconds after beginning stimulation.....	183	128
11:04 a.m.	Before sciatic stimulation (9 cm.).....	200	110
	20 seconds after beginning stimulation.....	209	122
	45 seconds after beginning stimulation.....	205	116
11:15 a.m.	Excised left adrenal (extraperitoneally).....		
11:16 a.m.	Before sciatic stimulation (10 cm.).....	182	83
	5 seconds after beginning stimulation.....	180	85
11:20 a.m.	Prepared central end of right sciatic.....		
11:27 a.m.	Before stimulation of left sciatic (8 cm.).....	165	80
	23 seconds after beginning stimulation.....	168	100
11:43 a.m.	Before stimulation of right sciatic (6 cm.).....	161	75
	2 seconds after beginning stimulation.....	160	
	6 seconds after beginning stimulation.....	164	
	17 seconds after beginning stimulation.....	170	94
	3 seconds after end of stimulation.....	171	

⁷ The eye reactions were the same as described in footnote to protocol of cat 190, p. 319.

		Rate	Pressure
11:46 a.m.	Before sciatic stimulation (6 cm.).....	160	76
	10 seconds after beginning stimulation.....	169	98
	6 seconds after end of stimulation.....	170	
11:50 a.m.	Before stimulation of left sciatic (6 cm.).....	155	62
	6 seconds after beginning stimulation.....	157	70
12:07 p.m.	Before stimulation of left sciatic (6 cm.).....	147	50
	7 seconds after beginning stimulation.....	151	
	30 seconds after beginning stimulation.....	153	56

In both of the experiments hitherto cited in this section (cats 440 and 443) the blood pressure after removal of the second adrenal remained good, practically unchanged in the first cat and only moderately lowered in the other. In the next experiment (cat 438) a considerable fall of pressure accompanied the operation for removal of the second adrenal. There is no reason to attribute this to the fact that the operation was done by the abdominal route. A similar fall of pressure occurred in cat 439 after removal of the second adrenal extraperitoneally by the lumbar route.

Protocol. Cat 438; female, weight 1.91 kgm. Right adrenal excised 3 days previously. Under urethane (3 gm.) cut vago-sympathetics, excised stellate ganglia, prepared central end of left sciatic for stimulation.

		Rate	Pressure
12:08 p.m.	Before sciatic stimulation (6 cm. to 4 cm.).....	189	122
	After increasing strength of stimulus (5 cm.).....	194	
	After increasing strength of stimulus (4 cm.).....	200	
	At end of stimulation.....	213	154
12:30 p.m.	Before sciatic stimulation (6-4 cm.).....	206	110
	4 seconds after beginning stimulation.....	220	140
	After increasing strength of stimulus (4 cm.).....	237	178
12:45 p.m.	Opened abdomen, excised left adrenal.....		
12:50 p.m.	Before sciatic stimulation (4 cm.).....	130	40
	During first 15 seconds stimulation.....	138	
	After increasing strength of stimulus.....	143	52
	After further increasing strength of stimulus.....	150	64
1:03 p.m.	Before sciatic stimulation (6-4 cm.).....	161	
	5 seconds after beginning stimulation.....	170	
1:10 p.m.	Before sciatic stimulation (6-4 cm.).....	161	66
	5 seconds after beginning stimulation.....	162	
	30 seconds after beginning stimulation.....	166	83
1:15 p.m.	Before sciatic stimulation (4 cm.).....	171	60
	10 seconds after beginning stimulation.....	170	74
1:20 p.m.	Before total asphyxia for 1 minute.....	175	64
	Counting from end of asphyxia.....	225	64

In cat 438 it should be noted that with the fall of pressure from well over 100 mm. to 40 mm. of mercury, associated with the adrenalectomy, the pulse rate diminished from 206 before the previous sciatic stimulation to 130. To attribute this diminution of 76 beats per minute entirely to the lack of the epinephrin from the second adrenal is, we believe, unwarranted. As the pressure gradually rose later on in the experiment, be it remembered in the absence of the adrenals, the pulse rate increased also to 161, 171 and 175 with pressures of 60 to 66. In spite of the rather low blood pressure which, however, showed a tendency to improve, a fair acceleration of the heart was elicited by stimulation of the sciatic after removal of the second adrenal (as much as 20 beats per minute, compared with 24 beats and 31 beats in two observations before the adrenalectomy).

Protocol. Cat 439; male; weight, 1.93 kgm. Right adrenal excised 5 days previously. Under urethane (3 gm.) cut vago-sympathetics, excised stellate ganglia and prepared central end of left sciatic for stimulation.

		Rate	Pressure
11:30 a.m.	Before sciatic stimulation (10 cm.).....	210	125
	4 seconds after beginning stimulation.....	217	146
	After increasing strength of stimulus (8 cm.).....	234	160
11:40 a.m.	Before sciatic stimulation (6 cm.).....	200	100
	3 seconds after beginning stimulation.....	222	142
11:50 a.m.	Excised left adrenal (extraperitoneally).....		
11:53 a.m.	Before sciatic stimulation (6 cm.).....	178	54
	15 seconds after beginning stimulation.....	181	68
	After increasing strength of stimulus (5 cm.).....	180	66
12:00 m.	Before sciatic stimulation (6 cm.).....	179	56
	6 seconds after beginning stimulation.....	183	72
12:10 p.m.	Prepared central end of right sciatic.....		
12:12 p.m.	Before stimulation of right sciatic (7 cm.).....	180	40
	5 seconds after beginning stimulation.....	183	58
	After increasing strength of stimulus (3 cm.).....	183	49
12:20 p.m.	Before sciatic stimulation (6 cm.).....	177	40
	5 seconds after beginning stimulation.....	182	44
	After end of stimulation.....	182	40
12:25 p.m.	Before intravenous injection of Ringer.....	173	20
	Immediately after injection of 100 cc. Ringer.....	188	46
	30 seconds after end of injection.....	190	68

In cat 439 although the blood pressure, immediately after removal of the second adrenal, was a little higher than in cat 438, it tended to grow progressively worse, sinking at last to 20 mm. of mercury, whereas the opposite tendency was seen in cat 438. Only very trifling accelerations of the heart were caused by sciatic stimulation after the adre-

nalectomy, although before fair reactions were obtained (as much as 24 beats in one observation and 22 beats per minute in another). The changes of blood pressure produced by stimulation of the sciatic were also small. Since the heart reaction depends upon a reflex or reflexes, as already pointed out, it necessarily fails or is diminished when the reflex arcs have deteriorated under the influence of a low blood pressure and a poor blood flow. Toward the end of the experiment an injection of Ringer's solution raised the blood pressure from 20 to 68 mm. and the pulse rate from 173 to 190. Epinephrin, of course, could have nothing to do with this acceleration. That the relative failure of the heart reaction on sciatic stimulation after removal of the second adrenal was not dependent upon the impossibility of a reflex increase in the epinephrin output but upon other, probably circulatory conditions, is indicated in the next experiment (cat 446), the last to be cited in this section.

Protocol. Cat 446; female; weight, 1.67 kgm. Right adrenal excised 6 days previously. Under urethane cut vago-sympathetics, excised stellate ganglia, prepared central end of right sciatic for stimulation.

		Rate	Pressure
11:00 a.m.	Before sciatic stimulation (8 cm.).....	185	115
	10 seconds after beginning stimulation.....	200	152
	20 seconds after beginning stimulation.....	203	
	30 seconds after beginning stimulation.....	204	123
11:02 a.m.	Before sciatic stimulation (9 cm.).....	185	104
	10 seconds after beginning stimulation.....	200	130
	32 seconds after beginning stimulation.....	199	113
11:20 a.m.	Excised left adrenal (extraperitoneally).....		
11:22 a.m.	Before sciatic stimulation (9 cm.).....	148	57
	10 seconds after beginning stimulation.....	150	64
	34 seconds after beginning stimulation.....	150	61
11:30 a.m.	Intravenous injection of 100 cc. Ringer.....		
11:50 a.m.	Before sciatic stimulation (7 cm.).....	164	80
	10 seconds after beginning stimulation.....	174	
	25 seconds after beginning stimulation.....	178	115
	40 seconds after beginning stimulation.....	173	
11:55 a.m.	Before sciatic stimulation (8 cm.).....	161	76
	4 seconds after beginning stimulation.....	163	
	22 seconds after beginning stimulation.....	168	97
	40 seconds after beginning stimulation.....	162	82
12:03 p.m.	Before sciatic stimulation (6 cm.).....	162	72
	5 seconds after beginning stimulation.....	170	88
	23 seconds after beginning stimulation.....	171	
	42 seconds after beginning stimulation.....	169	70
12:06 p.m.	Opened abdomen, tied renal arteries and veins.....		

		<i>Rate</i>	<i>Pressure</i>
12:11 p.m.	Clipped abdominal aorta and stimulated sciatic;		
	before stimulation (7 cm.)	171	74
	10 seconds after beginning stimulation.....	173	
	35 seconds after beginning stimulation.....	176	83
	After removal of clip.....	172	53
12:20 p.m.	Intravenous injection of 50 cc. Ringer.....		
12:25 p.m.	Clipped abdominal aorta and stimulated sciatic;		
	before stimulation (7 cm.)	170	90
	10 seconds after beginning stimulation.....	179	
	28 seconds after beginning stimulation.....	176	96
	After release of aorta.....	174	81
12:30 p.m.	Prepared central end of left sciatic for stimulation....		
12:32 p.m.	Clipped abdominal aorta and stimulated left sciatic;		
	before stimulation (7 cm.)	173	88
	10 seconds after beginning stimulation.....	180	
	30 seconds after beginning stimulation.....	185	110
	45 seconds after beginning stimulation.....	180	88
	10 seconds after release of aorta.....	174	63
12:35 p.m.	Clipped abdominal aorta and stimulated left sciatic;		
	before stimulation (6 cm.)	163	70
	10 seconds after beginning stimulation.....	169	78
	31 seconds after beginning stimulation.....	171	60
	10 seconds after release of aorta.....	166	45

After removal of the second adrenal the blood pressure fell from over 100 mm. of mercury to 57 mm. and the pulse rate dropped from 185 to 147 beats per minute. Sciatic stimulation caused practically no acceleration of the heart (3 beats per minute as compared with 15 beats before the adrenalectomy) and very little rise of blood pressure (7 mm.). The pressure went on falling and Ringer's solution was injected, which brought the blood pressure up to 80 mm. Stimulation of the sciatic nerve now caused an acceleration of 14 beats per minute and a rise of blood pressure of 35 mm. of mercury. Another stimulation caused an acceleration of 12 beats when the blood pressure had been raised to 88 mm. of mercury by temporary clipping of the abdominal aorta, the clip being put on and the pressure allowed to become constant, which only required a fraction of a minute, before the beginning of stimulation. Samples of portions of the tracings used for counting the pulse rate are given in figures 9 and 10, and the whole curves (much reduced) from which these portions were taken, in figure 11.

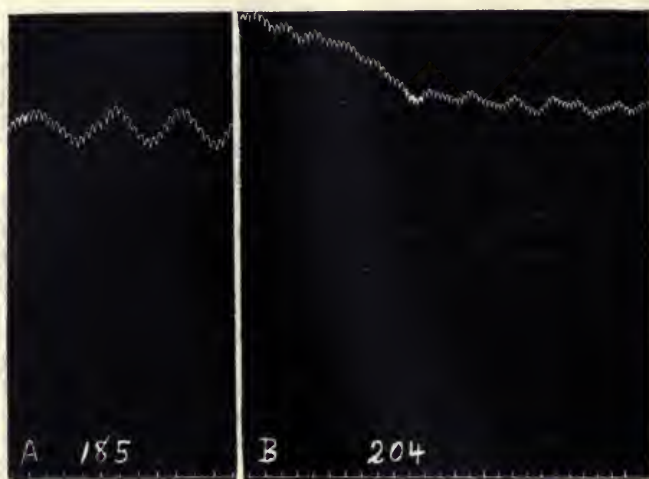


Fig. 9. Parts of blood pressure tracings from cat 446. *A*, before and *B*, a portion commencing 19 seconds after beginning of sciatic stimulation. Reduced to four-fifths.

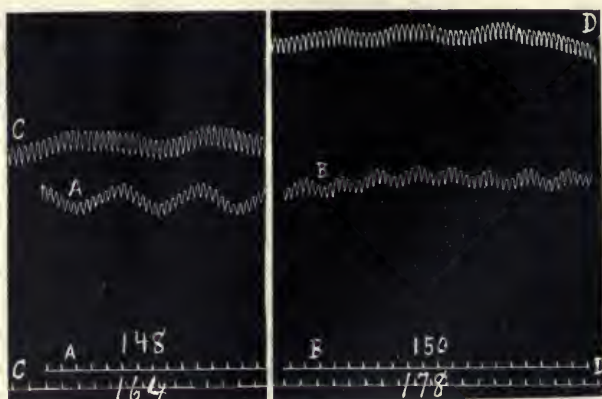


Fig. 10. Parts of blood pressure tracings from cat 446. *A*, before and *B*, a portion commencing 16 seconds after beginning of sciatic stimulation, after excision of remaining adrenal; *C*, before and *D*, 23 seconds after beginning of sciatic stimulation, after intravenous injection of Ringer. Reduced to four-fifths.

EXPERIMENTS IN WHICH BOTH ADRENALS WERE REMOVED AT ONE TIME

It seems probable that in most of the cats with one adrenal removed some time before the experiment the interval was too short for the full advantage of this procedure to be obtained, if there is an advantage. The results were, nevertheless, decisive as regards the question at issue. To check the matter a series of observations was made in which both adrenals were removed, as carefully as possible, at the time of the experiment on the denervated heart reaction. The protocol of cat 449 is cited as an example of experiments in which the adrenals were extirpated after opening the abdomen.

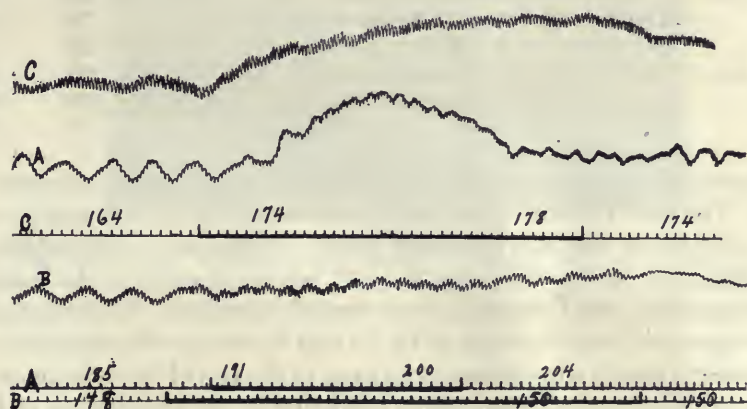


Fig. 11. Blood pressure curves from cat 446. A, sciatic stimulation before and B, after excision of remaining adrenal; C, after intravenous injection of Ringer. Reduced to one-half.

Protocol. Cat 449; male; weight, 3.38 kgm. Under urethane (6 gm.) cut vago-sympathetics, excised stellate ganglia, prepared central end of left sciatic for stimulation.

		Rate	Pressure
12:00 m.	Before sciatic stimulation (8 cm.).....	285	125
	10 seconds after beginning stimulation.....	293	170
	25 seconds after beginning stimulation.....	294	
12:12 p.m.	Before sciatic stimulation (6 cm.).....	256	110
	15 seconds after beginning stimulation.....	280	147
	40 seconds after beginning stimulation.....	283	130
12:35 p.m.	Opened abdomen, excised both adrenals.....		
12:36 p.m.	Before sciatic stimulation (6 cm.).....	228	70
	12 seconds after beginning stimulation.....	238	124
	35 seconds after beginning stimulation.....	238	90

		Rate	Pressure
12:50 p.m.	Before sciatic stimulation (5 cm.).....	223	74
	15 seconds after beginning stimulation.....	241	127
	30 seconds after beginning stimulation.....	238	89
1:05 p.m.	Before sciatic stimulation (7 cm.).....	222	73
	10 seconds after beginning stimulation.....	237	130
	30 seconds after beginning stimulation.....	234	92
1:15 p.m.	Prepared peripheral end of left splanchnic in thorax for stimulation.		
1:20 p.m.	Before splanchnic stimulation (9 cm.).....	226	58
	10 seconds after beginning stimulation.....	231	111
	30 seconds after beginning stimulation.....	223	69
1:25 p.m.	Before splanchnic stimulation (7 cm.).....	221	59
	8 seconds after beginning stimulation.....	229	110
1:40 p.m.	Before sciatic stimulation (6 cm.).....	221	52
	10 seconds after beginning stimulation.....	231	92
	26 seconds after beginning stimulation.....	230	
1:45 p.m.	Before sciatic stimulation (6 cm.).....	223	52
	12 seconds after beginning stimulation.....	232	98
	40 seconds after beginning stimulation.....	233	64

Two sciatic stimulations were made before extirpation of the adrenals. The first yielded a maximum acceleration of 9 beats only, but the initial heart rate was unusually great. In the second stimulation the maximum acceleration was 24 to 27 beats per minute, the initial rate being decidedly lower than in the first observation. After removal of the adrenals, accelerations of 10, 18 and 15 beats were obtained in 3 successive sciatic stimulations, and even at the end of the experiment, when the blood pressure had fallen considerably, an acceleration of 10 beats was gotten. It is scarcely necessary to point out that it would be futile to try to determine from such figures whether epinephrin was taking any sensible share in the reaction before the adrenalectomy, and if so, how much. For the maximum acceleration of which a given heart is capable at different stages in an experiment, under the influence of the changes produced by stimulation of the sciatic other than any possible change in the epinephrin output, must vary with the condition of the heart, and this with the blood flow on which its nutrition depends. Stimulation of the peripheral end of a splanchnic nerve after removal of both adrenals caused also distinct acceleration, as much as 8 to 10 beats per minute, in this experiment although, of course, some further fall of blood pressure had been caused by division of the nerve. Portions of the curves used for counting the heart rate in the last sciatic stimulation prior to removal of the adrenals and the second stimulation after removal are given in figure 12, and a reduction

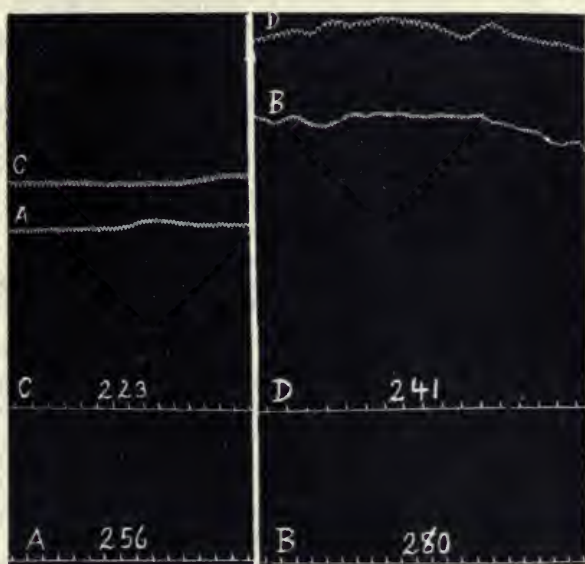


Fig. 12. Parts of blood pressure tracings from cat 449. *A*, before and *B*, a portion commencing 15 seconds after beginning of sciatic stimulation, before excision of both adrenals; *C*, before and *D*, 15 seconds after beginning of sciatic stimulation, after excision of both adrenals. Reduced to four-fifths.

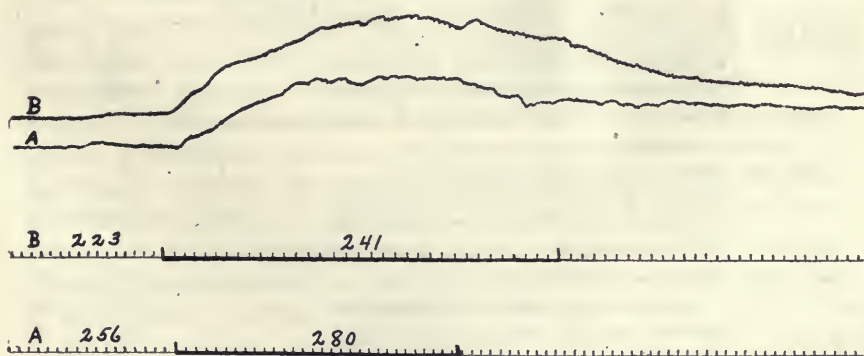


Fig. 13. Blood pressure curves from cat 449. *A*, sciatic stimulation before and *B*, after excision of both adrenals. Reduced to one-half.

of the greater part of the two curves in figure 13. It will be seen that neither the blood pressure reaction nor the acceleration was essentially modified by the absence of the adrenals. The blood pressure, although decidedly lower after the adrenalectomy, was still good (70 to 80 mm. of mercury, as compared with 110 mm. before the operation).

In the next experiment (cat 448) the adrenals were removed extra-peritoneally by the lumbar route. The results were practically the same as in cat 449, in which the abdomen had been opened.

Protocol. Cat 448; male; weight, 3.29 kgm. Under urethane (6 gm. in two doses) cut vago-sympathetics, excised stellate ganglia, prepared central end of left sciatic for stimulation.

		Rate	Pressure
11:43 a.m.	Before sciatic stimulation (10 cm.).....	217	132
	10 seconds after beginning stimulation.....	226	158
	30 seconds after beginning stimulation.....	232	144
11:45 a.m.	Before sciatic stimulation (8 cm.).....	210	135
	10 seconds after beginning stimulation.....	234	170
	30 seconds after beginning stimulation.....	242	149
12:20 p.m.	Extirpated both adrenals (extraperitoneally).....		
12:25 p.m.	Before sciatic stimulation (8 cm.).....	165	90
	6 seconds after beginning stimulation.....	171	103
	25 seconds after beginning stimulation.....	170	
12:32 p.m.	Before sciatic stimulation (7 cm.).....	164	87
	10 seconds after beginning stimulation.....	181	132
	26 seconds after beginning stimulation.....	184	128
	35 seconds after beginning stimulation.....	182	118
12:40 p.m.	Sciatic now stimulated twice with an interval of only 2 minutes, to fatigue the reaction, and then at		
12:50 p.m.	Before sciatic stimulation (6 cm.).....	173	92
	10 seconds after beginning stimulation.....	181	126
	35 seconds after beginning stimulation.....	183	
1:03 p.m.	Sciatic again stimulated twice in rapid succession and then at.....		
1:14 p.m.	Before sciatic stimulation (6 cm.).....	171	76
	10 seconds after beginning stimulation.....	175	107
	40 seconds after beginning stimulation.....	177	
1:20 p.m.	Opened abdomen, tied renal arteries and veins.....		
1:24 p.m.	Before sciatic stimulation (6 cm.).....	174	88
	4 seconds after beginning stimulation.....	181	110
	30 seconds after beginning stimulation.....	181	98
1:33 p.m.	Stimulated sciatic with abdominal aorta clipped. Before clipping aorta.....	170	77
	Before sciatic stimulation.....	177	100
	10 seconds after beginning stimulation.....	184	
	30 seconds after beginning stimulation.....	186	114
	After release of aorta.....	184	
	30 seconds after release of aorta.....	185	86

1:37 p.m.	Stimulated sciatic with abdominal aorta and vena cava clipped;		
	Before stimulation (7 cm.).....	Rate 174	Pressure 76
	8 seconds after beginning stimulation.....	180	
	30 seconds after beginning stimulation.....	182	100
	10 seconds after removal of clips from aorta and cava.	176	68
2:21 p.m.	Before sciatic stimulation (7 cm.).....	159	47
	10 seconds after beginning stimulation.....	159	60
	Just after end of stimulation.....	158	
2:25 p.m.	Before sciatic stimulation (4 cm.).....	155	43
	10 seconds after beginning stimulation.....	161	55
	40 seconds after beginning stimulation.....	161	

Accelerations as great as 20 beats per minute were obtained after removal of the adrenals. Before removal the maximum acceleration seen in two observations with different strengths of stimulus was 15 and 32 beats respectively. The blood pressure fell from 135 to 90 mm. of mercury after the adrenalectomy. When the sciatic was then repeatedly stimulated with only short intervals between the successive stimulations, the heart reaction, as estimated by the maximum acceleration, diminished but about the same absolute rate (180 to 183) was reached at the height of the acceleration. Later on in the experiment, when the abdomen had been opened and the renal vessels tied, an acceleration of 7 beats per minute was caused by clipping the abdominal aorta just above the bifurcation, as is done in the collection of blood from the cava pocket. This acceleration was accompanied by a rise of blood pressure from 77 to 100 mm. of mercury. When the sciatic was now stimulated with the aorta still clipped (the clip was put on only a short time before stimulation of the sciatic so that the excitation of the nerve should not be interfered with) a further acceleration of 9 beats a minute occurred. Sciatic stimulation gave a similar acceleration with both abdominal aorta and cava clipped, as in making the pocket. At the end of the experiment, when the blood pressure had fallen to 40 to 50 mm. of mercury, no sensible acceleration, or with stronger stimulation only a small one, could be elicited through the sciatic and the rise of blood pressure was also small. But it would surely be absurd to attribute this belated failure of a reaction which had been well obtained after the adrenalectomy to the absence of a reflexly excited outpouring of epinephrin.

As to varying the strength of stimulation, it should be stated that we did not consider there would be any point in merely comparing the reaction obtained before and after the removal of the adrenals with

practically the same nominal strength of stimulus, since the excitability of the reflex mechanisms cannot be counted upon to remain the same, especially where considerable changes of blood pressure have occurred. What we tried to do in all the experiments was to elicit as large a reaction as possible, both before and after elimination of the adrenals; using for this purpose the strength of stimulation which seemed most effective. The strongest stimuli were not generally the best. Not infrequently we found that stimuli of the same strength as gave the maximum acceleration before removal of the adrenals did so after their removal also. But sometimes it was necessary to increase the stim-

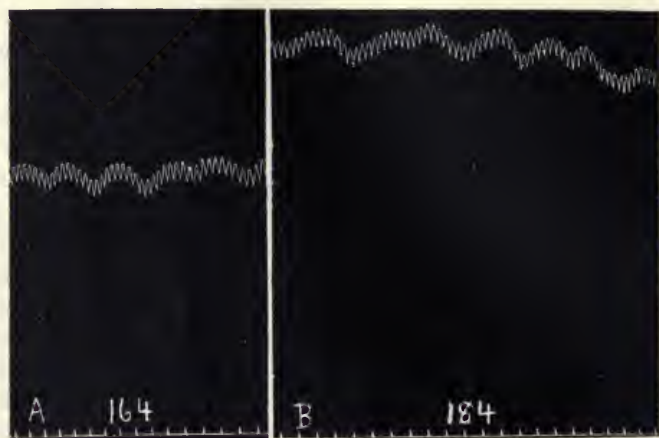


Fig. 14. Parts of blood pressure tracings from cat 448. *A*, before and *B*, a portion commencing 26 seconds after beginning of sciatic stimulation, after excision of both adrenals. Reduced to four-fifths.

ulus. This is mentioned because if the same strength of stimulus is employed before and after removal of the adrenals, a small, or even no acceleration might be obtained after the operation, which would not mean that the reaction had disappeared because of the loss of the adrenals, but that the excitability of the mechanisms concerned in it had diminished. The same is, of course, true of the vasomotor reflex, which we took as an indicator of effective stimulation.

Portions of a curve from cat 448, showing acceleration on sciatic stimulation after removal of the adrenals, are reproduced in figure 14, and the greater part of the curve on a reduced scale in figure 15.

Protocol. Cat 437; female; weight, 1.61 kgm. Left superior cervical ganglion excised one month previously. Under urethane (3 gm.) cut vago-sympathetics, excised stellate ganglia, prepared central end of left sciatic for stimulation.

		Rate	Pressure
11:46 a.m.	Before sciatic stimulation ⁸	260	116
	5 seconds after beginning stimulation.....	274	150
11:50 a.m.	Opened abdomen, tied off renal arteries and veins.....		
11:55 a.m.	Before sciatic stimulation.....	253	60
	10 seconds after beginning stimulation.....	264	84
12:00 m.	Before stimulation of sciatic with abdominal aorta clipped.....	250	64
	2 seconds after beginning stimulation.....	270	88
	15 seconds after beginning stimulation.....	273	
12:05 p.m.	Before stimulation of sciatic with abdominal aorta and cava clipped.....	255	60
	4 seconds after beginning stimulation.....	278	84
12:10 p.m.	Excised both adrenals.....		
12:11 p.m.	Before sciatic stimulation.....	203	44
	4 seconds after beginning stimulation.....	232	59
2:15 p.m.	Before sciatic stimulation.....	214	42
	10 seconds after beginning stimulation.....	225	53

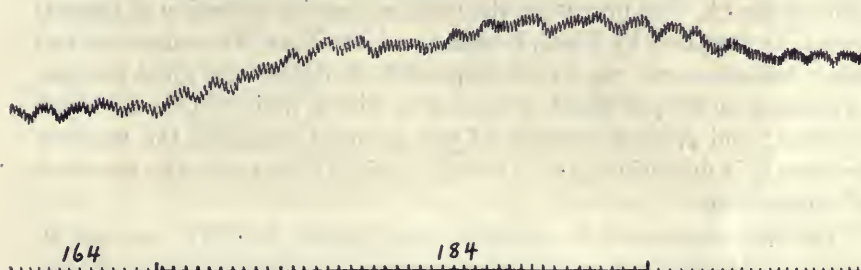


Fig. 15. Blood pressure curve from cat 448. Sciatic stimulation after excision of both adrenals. Reduced to one-half.

In cat 437, in which a superior cervical ganglion had been excised a month previously and in which, therefore, eye reactions were also available, the adrenalectomy was performed at a different stage of the experiment, and after the abdomen had been opened for some time and observations made on the results of sciatic stimulation with the abdominal aorta and cava clipped, as in the collection of blood from the adrenals. The demonstration that the heart reaction is obtained after removal of the adrenals was, if anything, more striking than in many of the other experiments. Sciatic stimulation yielded a maximum

⁸ The eye reactions were those described in the footnote to protocol of cat 190, p. 319.

acceleration of 14 beats per minute before the abdomen was opened. After opening the abdomen and tying off the renal vessels, the blood pressure had fallen to 60 mm. of mercury as compared with 116 mm. at the beginning of the experiment. An acceleration of 11 beats was given by stimulation of the sciatic. The abdominal aorta was now clipped and a short interval allowed for any effect on blood pressure and heart rate to develop, and then the sciatic was stimulated. The heart rate compared with that immediately before stimulation was increased by 23 beats per minute. An equal acceleration was given in another observation, in which the abdominal aorta and inferior cava (above the junction of the iliac veins) were clipped in the same way shortly before stimulation. In such observations the clips were not removed till the portion of the curve to be used for counting the heart beats had been completed. Both adrenals were then excised and thereafter an acceleration of 29 beats was caused by stimulation of the sciatic. It is surely impossible to reconcile the results of such an experiment with Cannon's statements: *a*, that after opening the abdomen it is very rare to obtain any acceleration of the heart by sciatic stimulation; *b*, that preparing the cava pocket for collection of adrenal blood, as practised by Biedl, Hoskins and McClure (14), ourselves and other investigators, renders it impossible to detect the great increase in epinephrin output which according to him is indicated by the heart reaction; and *c*, that removal of the adrenals abolishes the reaction because it is due entirely to a reflexly excited increase in the secretion of epinephrin.

The last experiment to which we shall allude (cat 447) was one in which sciatic stimulation caused only a small acceleration at the beginning of the experiment, accompanied by insignificant changes of blood pressure. The cat was only half grown, but it is not known whether this had anything to do with the relatively small effects. The reaction was not essentially modified by removal of the adrenals, or subsequently by clipping the abdominal aorta. The blood pressure was diminished from about 100 mm. to little over 50 mm. of mercury after the adrenalectomy, and the heart rate was also diminished from over 200 to 175 beats a minute. As the blood pressure fell still lower toward the end of the experiment, the heart rate diminished further to 160 beats a minute.

Protocol. Cat 447; female; weight, 1.24 kgm. Under ether cut vago-sympathetics; excised stellate ganglia; prepared central end of left sciatic for stimulation.

		Rate	Pressure
11:00 a.m.	Before sciatic stimulation (8 cm.).....	203	115
	Just after end of stimulation.....	218	110
11:02 a.m.	Before sciatic stimulation (10 cm.).....	210	100
	12 seconds after beginning stimulation.....	207	100
11:05 a.m.	Before sciatic stimulation (9 cm.).....	205	110
	10 seconds after beginning stimulation.....	209	112
11:11 a.m.	Before sciatic stimulation (7 cm.).....	206	110
	12 seconds after beginning stimulation.....	209	118
	20 seconds after beginning stimulation.....	212	
11:18 a.m.	Before sciatic stimulation (9 cm.).....	202	104
	15 seconds after beginning stimulation.....	208	108
11:40 a.m.	Opened abdomen, excised both adrenals		
11:43 a.m.	Before sciatic stimulation (7 cm.).....	175	53
	15 seconds after beginning stimulation.....	181	63
	40 seconds after beginning stimulation.....	184	
11:46 a.m.	Before sciatic stimulation (9 cm.).....	179	55
	15 seconds after beginning stimulation.....	182	55
	30 seconds after beginning stimulation.....	183	55
11:48 a.m.	Before sciatic stimulation (5 cm.).....	172	54
	Just after beginning stimulation.....	177	
	10 seconds after beginning stimulation.....	172	54
11:53 a.m.	Before stimulation of sciatic with abdominal aorta clipped (8 cm.).....	172	55
	6 seconds after beginning stimulation.....	171	59
	After end of stimulation.....	179	
12:10 p.m.	Before stimulation of sciatic with abdominal aorta clipped (7 cm.).....	160	49
	6 seconds after beginning stimulation.....	160	51
	25 seconds after beginning stimulation.....	166	
12:15 p.m.	Before sciatic stimulation (8 cm.).....	163	35
	10 seconds after beginning stimulation.....	166	48
	30 seconds after beginning stimulation.....	164	

The slowing of the denervated heart after interference with the epinephrin output. It is not easy to demonstrate conclusively that elimination of the epinephrin output of the adrenals causes a slowing of the denervated heart, indicating that the ordinary output, under the conditions of our experiments, is capable of exerting an influence upon the heart. For when the elimination of the epinephrin output is brought about by removal of the adrenals in an acute experiment, the result may be complicated by a slowing associated with a fall of blood pressure. Nevertheless, as stated previously (15), "evidence of a relation of the

normal epinephrin output to the heart rate seems to be afforded by a comparison of the effects produced in cats on the rate by excision of the stellate ganglia after previous section of the vagi when the adrenal epinephrin is normally entering the circulation and in the absence of epinephrin." This is illustrated in table 1.

Although the number of animals is small, the results are suggestive. In the three cats which had been subjected to the adrenal operation mentioned there was a decided diminution in the heart rate after removal of the ganglia. In cat 235 the rate was unusually slow

TABLE 1

CAT	BEFORE		AFTER REMOVAL OF STELLATES		REMARKS
	Rate	Pres- sure	Rate	Pres- sure	
231	216	176	(a)152 (b)164	104 82	33 days after adrenal operation (b) 10 minutes later than (a)
233	193	87	168	76	36 days after adrenal operation
235	142	106	(a)144 (b)129	90 78	34 days after adrenal operation (b) 2 minutes later than (a)
234	266	152	255	122	
236	250	184	(a)249 (b)233	168 118	(b) 7 minutes later than (a)
237	192	126	(a)187 (b)178	110 104	(b) 4 minutes later than (a)
193	172	120	188	114	{ Stellates removed an hour after beginning of experiment after repeated splanchnic stimulation
195	181	78	145	52	

In the first three cats the right adrenal had been removed and the nerves of the left cut. The last 5 cats were normal animals. All were anesthetized with urethane.

(142 beats per minute) before the excision of the stellate ganglion and immediately after excision of the second ganglion it was 144. But it at once began to fall and in 2 minutes was 129. An acceleration is not infrequently seen during the operation for removal of the ganglia, due it may be supposed to reflex stimulation of the accelerantes or direct mechanical stimulation or possibly to indirect effects produced through vasomotor changes. The full effect of the elimination of the accelerator nerves may then not be seen for a minute or two.

In cat 231 the epinephrin output as estimated on adrenal vein blood by assay on rabbit intestine segments could not have been one-twenty-

fifth of the normal average. In cat 233 it could not have been more than one-twentieth of the normal average (intestine and pupil assay), and in cat 235 it could not have been one-three-hundredth of the normal; even the adrenal blood sample with the slowest flow (0.6 gm. per minute) was shown by the intestine segment test to have a smaller concentration than 1:300,000,000 adrenalin. In the five normal cats in table 1 only in one was a decided slowing of the heart observed after excision of the stellate ganglia and this was associated with a marked fall of blood pressure.

TABLE 2

CAT	BEFORE		AFTER REMOVAL OF REMAINING ADRENAL		Days after operation
	Rate	Pressure	Rate	Pressure	
441	219	100	214	86	7
444	131	141	137	138	8
450	173	102	174	94	34
438	195	104	(a)195*	74	3
439	210	125	(b)130	40	
440	226	134	178	54	5
443	180	116	212	124	6
445	161	128	182	83	7
446	185	115	143	86	5
			147	57	6
			164	80†	

In the first three cats the right adrenal had been excised and the nerves of the left cut. In cat 445 the left and in the others the right adrenal had been removed. Cats 440, 441, 443, 444, 445 were etherized, the others were under urethane.

* (a) immediately and (b) 4 to 5 minutes after removal of adrenal. The blood pressure was falling continuously during this time.

† After injection of Ringer's solution.

In table 2 are shown the heart rates and blood pressures before and after removal of the remaining adrenal from three cats in which the right adrenal had been excised and the nerves of the left cut, and in seven cats from which one adrenal had been removed at a previous operation. The vago-sympathetics had been cut and the stellate ganglia excised at the beginning of the experiment. In the three cats whose remaining adrenal had been denervated no diminution in the pulse rate was caused by excision of the gland. As already remarked, the fact that the blood pressure was practically unaltered by removal

of the previously denervated adrenal in these cats makes it difficult to estimate the influence of the suppression of the epinephrin output from the gland upon the result. In four out of the other seven cats a substantial slowing of the heart was found after removal of the remaining adrenal. In another of the seven cats (438) although the rate was unchanged immediately after removal of the remaining adrenal the blood pressure was steadily falling and 5 minutes later the pulse rate was only 130.

In table 3 are given the heart rates and blood pressures in five normal cats before and after removal of both adrenals at one operation, the vago-sympathetics having been cut and the stellate ganglia excised at the beginning of the experiment. In every case there was a diminu-

TABLE 3

CAT	BEFORE		AFTER REMOVAL OF BOTH ADRENALS		ANESTHETIC
	Rate	Pressure	Rate	Pressure	
447	203	115	175	53	Ether
448	218	132	165	90	Ether
449	285	125	228	70	Urethane
437	256	116	203	44	Urethane
195*	146	44	131	38	Urethane

* In cat 195 the adrenals were removed about an hour after removal of stellates. The rate just before removal of the adrenals was 145.

tion in the heart rate, but this again was in every case associated with a fall of blood pressure.

Table 4 shows the heart rates and blood pressures in fourteen normal cats after section of the vago-sympathetics and excision of the stellate ganglia. In three dogs anesthetized with morphine and ether the pulse rates after denervation of the heart were 106, 153 and 173, beats per minute, with blood pressures of 92, 72 and 38 mm. of mercury respectively. That different anesthetics may affect the pulse rate of the denervated heart differently may be assumed, e.g., chloroform diminishes the rate in dogs according to v. Anrep (1). So far as can be judged from a relatively small number of observations in our own experiments, the difference between ether and urethane was not conspicuous.

If all the results on the cats are brought together, some further suggestive points seem to emerge. Thus, in twelve out of twenty⁹ normal cats with both adrenals intact the pulse rate was over 200 a minute after denervation of the heart. In two out of seven cats with one adrenal previously removed and in only two out of nine cats with one adrenal previously removed and the nerves of the other cut, was the pulse rate over 200 after denervation of the heart. The average pulse rate of the twenty cats with both adrenals intact, after denervation of the heart, was 209 beats per minute; the average for the nine cats whose epinephrin output had been previously interfered with by excision of one adrenal and section of the nerves of the other 170 beats

TABLE 4

CAT	RATE	PRESSURE	CAT	RATE	PRESSURE
201	177	54	190	250	123
202	145	92	191	240	124
215	219	134	196	212	72
175	188		198	233	60
	176	82*	199	137	113
176	160		200	210	84
177	185	132	436	203	56
179	169	80			

* 2 hours after the first observation.

In cats 201, 202 and 215 the right adrenal had been excised and the nerves of the left cut 18 days, 8 days and 59 days respectively before the operation, the others were normal cats. All were anesthetized with urethane, cat 436 more deeply than the rest.

per minute; and the average for the seven cats from which one adrenal had been previously removed 184 beats per minute. A possible influence of the previous operation as such, apart from interference with epinephrin output, where the interval was only a few days is not excluded, but is not discernible in the tables.

⁹ In 4 additional normal cats the heart rates and blood pressures after denervation of the heart, but before adrenalectomy, were 266 (214), 284 (145), 265 (145), and 150 (66). In the last cat the abdomen had been opened before the heart was denervated. After removal or ligation of both adrenals the corresponding numbers were 222 (114), 283 (102), 255 (130), and 143 (57). In a fifth cat the rate after denervation of the heart was 248 and the blood pressure 150 mm. of mercury.

DISCUSSION AND SUMMARY

It has been shown by us that the acceleration of the heart caused by stimulation of the central end of the sciatic is in no way a reaction by which the rate of output of epinephrin from the adrenals can be estimated, or changes in that rate demonstrated, as claimed by Cannon. This is proved by the following facts:

a. Clipping of the adrenal veins has no demonstrable influence upon the occurrence and magnitude of the heart reaction caused by sciatic stimulation, although it markedly diminishes or abolishes reactions which are known to be genuine reactions for epinephrin, such as the dilatation of the pupil following stimulation of the peripheral end of a splanchnic nerve.

b. Acceleration of the denervated heart on sciatic stimulation is well obtained in cats which have been allowed to survive after removal of one adrenal and section of the nerves of the other, an operation which is known to abolish or greatly diminish the epinephrin output. In such cats the reaction is still elicited after the remaining adrenal has been removed.

c. Good acceleration of the heart can be elicited by stimulation of the sciatic in cats from which both adrenals have been removed, either in two operations with an interval for recovery interposed, or at one operation.

d. When the reaction disappears after removal of the adrenals this is not because of the absence of increased epinephrin discharge on stimulation of the sciatic but for other reasons, such as deterioration in the condition of the animal (fall of blood pressure, etc.) which interferes with the reflex or reflexes necessarily involved in the reaction or with the capacity of the heart to markedly accelerate its beat.

e. Contrary to Cannon's statement, the reaction is well obtained after opening the abdomen. It can be elicited after ligation of the renal vessels, abdominal aorta and inferior cava, as practised in forming a cava pocket for collection of adrenal vein blood. If the reaction indicates a greatly increased output of epinephrin reflexly induced by stimulation of the sciatic, as assumed by Cannon, we could not have failed to detect the increase by the direct method of collecting adrenal vein blood and assaying its epinephrin content on rabbit segments. But our results were negative (16).

As regards the real mechanism of the acceleration of the denervated heart caused by sciatic stimulation, we desire to point out, once for

all, that the onus of explaining this probably complex indirect reaction, which Cannon erroneously interprets as indicating increased epinephrin secretion, does not rest upon us at all. It is for Doctor Cannon to exclude, if he can, by control experiments, other possible factors in the reaction which he attributes solely to epinephrin. Our position is simply this. We have investigated the influence of stimulation of the sciatic and brachial nerves upon the rate of epinephrin output by a direct method, correct in principle and free from ambiguity, and have obtained negative results. Doctor Cannon states that by means of an indirect method (the denervated heart reaction) he obtains a positive result. We show that this reaction cannot yield any information as to the rate of epinephrin output from the adrenals or as to changes in that rate, since it is obtainable when the epinephrin output of the adrenals is abolished. And here we are entitled to rest our case, not, of course, claiming that sensory stimulation *cannot* increase the epinephrin output, but that no increase has hitherto been proved.

However, certain fairly obvious suggestions may be made as to factors which may play a part in the heart reaction under discussion, a reaction probably made up of more than one component. One is the larger amount of epinephrin sent through the coronary circulation and perhaps the greater concentration of it, owing to the vasomotor changes produced by stimulation of the sciatic (11), (15). It may be pointed out that even if Cannon's statement that the reaction cannot be obtained in the absence of the adrenals had been found correct, that of itself would only have shown that epinephrin is essentially concerned. For it might be due to the redistribution of the epinephrin without any increase in the rate of output.

Cannon attempts to invalidate this suggestion by an experiment in which he prevents a rise of pressure in the carotid during sciatic stimulation by compression of the chest, and yet obtains an acceleration of the heart. Now this is a quite complex experiment, and Doctor Cannon is doing several other things which may affect the heart besides keeping the pressure in the aorta constant. One of the things he is doing is impeding the venous return. The epinephrin, even if its rate of output remains unchanged, must, therefore, be diluted with a smaller proportion of indifferent blood in the right heart. Blood with a greater concentration of epinephrin must accordingly be passing through the coronaries, and as the concentration will increase in the same measure as the slowing in the venous return necessary to prevent rise of pressure, approximately the same amount of epinephrin will pass through the coronaries per unit of time as with a similar sciatic stimulation without compression of the chest. If then the ordinary output of epinephrin was a factor in the acceleration without compression it may be expected to exert the same influence during compression. In other words, an increase in the amount of epinephrin passing per unit of time through the coronary circulation during sciatic stimulation is not prevented by compressing the chest, so as to keep the blood pressure in the aorta from rising,

even if no increase in the rate of output of epinephrin has occurred, and the experiment is without significance for the question at issue. That no further acceleration occurred when the blood pressure rose after releasing the chest, is also just as intelligible, so far as epinephrin is a factor, on the assumption that the epinephrin output was not increased as on the assumption that it was increased by stimulation of the sciatic. For if the epinephrin concentration remained unchanged and the coronary blood flow was increased on decompressing the chest, an increased amount of epinephrin would pass through the coronaries per minute whether the output had been augmented by stimulation of the sciatic or not. The fact is, however, that with the increase in the venous inflow to the heart the concentration of epinephrin in the blood of the right heart must be proportionally diminished. But why, in any case, should a further increase in the heart rate have been expected, since the acceleration was already 36 beats a minute, the same as without compression of the chest? As there is no possibility that epinephrin is the sole factor in the heart reaction, as elicited reflexly, the point need not be labored. Whatever other factors are ordinarily concerned in the reaction, apart from the rise of arterial blood pressure, may be expected to act as well during compression of the chest as before. The possibility that a special factor, the gross interference with the mechanism of the heart, especially the filling and pressure of the right side and with the respiration might exert an influence in this experiment is not excluded.

We ourselves made two experiments (cats 198, 199) in which the rise of pressure on sciatic stimulation was largely prevented by hemorrhage, controlled by a mercury valve. The animals were anesthetized with urethane. In cat 198 before sciatic stimulation the pulse was 225, the blood pressure 78; during stimulation the pulse rate rose to 253 and the blood pressure to 192 mm. of mercury. In the next observation the pulse was 233 and the blood pressure 60 before stimulation. During stimulation the blood pressure was prevented from rising beyond 90 mm. of mercury, and the pulse rate increased only to 242 beats per minute. Blood (mixed with salt solution) was reinjected. Before stimulation of the sciatic the pulse rate was 272, the pressure 96. During stimulation the pulse rate rose to 286 with a pressure of 168 mm. of mercury. The sciatic was now stimulated while the pressure was prevented, by hemorrhage, from changing (it rose from 88 to 92). The pulse rate before stimulation was 258 and during stimulation 259. This experiment might seem to show that the acceleration was largely prevented by keeping the blood pressure from rising. But in the other cat a different result was obtained. In cat 199 the following pulse rates and blood pressures, the latter in parentheses, were recorded. Before stimulation 137 (113), during stimulation 215 (196). Before stimulation 130 (108), during stimulation with hemorrhage 193 (116-90). Before stimulation 144 (100), during stimulation 206 (162). Before stimulation 145 (102), during stimulation with hemorrhage 194 (115). Only these two experiments were made. For on reflection it was seen that this method also could not lead to any definite conclusion. If the epinephrin were the only factor in the acceleration, its concentration in the blood coming to the heart would increase as the mass of the circulating blood diminished, even provided that no increase were taking place in the rate of output.

Besides the direct accelerating action of epinephrin upon the heart there is another way in which the normal output of epinephrin may possibly play a part

in the acceleration caused by stimulation of the sciatic, by sensitizing the heart to the action of other factors, such as a rise of blood pressure, which in the absence of epinephrin might not be so effective. In this connection we recall the observation of v. Anrep (1) on the influence of adrenalin upon the power of the heart to adapt itself by changes in its tone to changes in the arterial pressure.

Whatever share epinephrin may take in the acceleration reaction it cannot be the only factor and is probably not the most important one, since excellent heart reactions can be obtained in the absence of the adrenals, provided that good vascular reflexes, as evidenced by the change of blood pressure, are elicited. The most obvious of the changes caused by stimulation of the central end of the sciatic, the rise of blood pressure, is the one which seems to be most intimately related to the heart acceleration. Since this relation is seen after, as before, elimination of the adrenals, the most direct suggestion is that the better blood flow through the coronary circulation is an important factor in the acceleration, either by raising the nutritive condition and the excitability of the mechanism in which the beat originates or by acting upon a local accelerator mechanism. An action of the increased blood pressure as such is not excluded. Indeed, long ago Johansson (17) pointed out that the acceleration of the heart (after section of the vago-sympathetics and excision of the stellate ganglia) caused by stimulation of the peripheral end of a splanchnic nerve and of the cut cervical cord, was dependent on the abruptness of the rise of pressure. At the time of Johansson's work, nothing was known of the secretory innervation of the adrenals, and it might be asked whether the whole acceleration in his experiments was not due to increased epinephrin output. This question, in our opinion, must be answered in the negative. For stimulation of the cord with one splanchnic cut produced in general a much greater acceleration than stimulation of one splanchnic, without any obvious reason why it should have caused a greater liberation of epinephrin, and this greater acceleration was accompanied by a larger rise of blood pressure. Further the dogs were curarized and curara (18) depresses the conductivity of the efferent epinephrin secretory path. We do not, however, know how much importance should be attached to this, as our work with curara was done on cats and was concerned only with the spontaneous liberation of epinephrin.

It is significant that the accelerations observed by him, taken in relation to the blood pressure changes, are of the same order of magnitude as those obtained by us with splanchnic and sciatic stimulation, whether with the adrenal veins clipped or in the absence of the adrenals. From our own observations, recorded in the preceding pages, it cannot be doubted that a rise of pressure, caused by stimulation either of the sciatic or the peripheral end of the splanchnic nerve, is associated with an acceleration of the denervated heart, unrelated to any immediate action of epinephrin. The maximum acceleration, as in Johansson's observations, may not be reached till the blood pressure has again begun to decline.

We agree with Johansson and with Lehndorff (19) that the manner in which the rise of pressure is produced is important as regards its action upon the heart. The latter observer remarks that raising the pressure by compressing the aorta seldom causes an acceleration, and that when acceleration is caused it is only slight. He was never able by inducing an asphyxial rise of pressure to elicit

the characteristic changes in the heart's action which he found on splanchnic stimulation. Guthrie and Pike (20) also found compression of the aorta inefficient. We have seen a definite acceleration, e.g., in cat 190 (see protocol), clipping the aorta in the thorax increased the pulse rate from 249 to 260 beats a minute, but this was a much smaller acceleration than that caused by splanchnic stimulation just before, although the increase of arterial pressure was greater when the aorta was clipped. In other cases we have seen no acceleration when the aorta was clipped in the thorax. Clipping of the abdominal aorta has been sometimes seen to cause some acceleration with a moderate rise of pressure, in the absence of the adrenals. The character of the blood sent to the heart is, not the same when the thoracic as when the abdominal aorta at the bifurcation is clipped, the liver and other viscera not being interfered with in the latter case. Apart altogether from the possible effect of epinephrin when the splanchnic is stimulated, the mechanical conditions under which the heart works are very different when the aorta is clipped and when the central end of the sciatic or the peripheral end of the splanchnic is stimulated, particularly as regards the venous inflow.

Cannon seems to criticise us for quoting the experiments of Guthrie and Pike on the influence of increased pressure of the perfusion fluid in accelerating the excised heart, as if this represented the sum total of our knowledge. We quoted this work, which was done in the laboratory of one of us, in a footnote to another paper (11), to show, and we believe it does show, that under certain conditions the heart, deprived of extrinsic innervation, can respond to changes of blood pressure by very considerable changes in frequency. When we stated that there is nothing strange about an increase in the rate of the denervated heart *in situ* when the central end of the sciatic or the peripheral end of the splanchnic is stimulated, and went on to say that it is obviously dependent upon the better blood flow through the coronary vessels, we had before us much of the evidence given on preceding pages that marked acceleration could be produced by stimulation of both of these nerves, in the absence of any possible output of epinephrin from the adrenals, and that the acceleration was associated with a rise of arterial pressure. Of the results of other observers on the heart *in situ*, we had in mind particularly the elaborate paper of Johansson (17) already referred to. The experiments of Martin and of Knowlton and Starling on the heart-lung preparation, which Cannon cites, were familiar to us, but we saw no point in quoting in a footnote observations which threw no light upon the accelerations we were obtaining by stimulation of the sciatic, under conditions which eliminated the liberation of epinephrin from the adrenals. Of course, the vasomotor reactions expressed in the rise of blood pressure under discussion might possibly have other actions upon the denervated heart, in addition to their most obvious action, the increased blood flow through the coronaries. It has been pointed out by various writers that it is not the same thing, as regards the heart rate, whether changes are induced in the arterial or in the venous pressure.

In the case of sciatic stimulation it might still be argued that the rise of pressure is only a sign that the stimulus is effective for some other reflex or reflexes on which the acceleration depends essentially. This is theoretically true, but any reflex which affects the composition of the blood, as sciatic stimulation may do (by causing hyperpnoea, increased reflex muscular action, possibly increased

mobilization of sugar or other changes in the liver through the splanchnics), must affect the heart concomitantly with the vasomotor reflex which increases the blood flow through the coronaries. In any case, the experiments on stimulation of the splanchnic in the absence of epinephrin output indicate the rise of arterial pressure as the most obvious of the factors associated with the heart reaction studied.

In two cats (one normal, 447, and one in which the epinephrin output had been interfered with, 444, both etherized), we have seen an acceleration, when sciatic stimulation elicited no rise of pressure either before or after elimination of the adrenals. But then there was evidence of effective stimulation, not only in the increased respiratory movements and respiratory blood pressure waves but also in a small depression of the blood pressure, succeeded by some rise after stoppage of the stimulation. In such a case the question presents itself, whether it is certain that in every individual the heart is completely severed from the central nervous system by section of the vagi and excision of the stellate ganglia. We repeat that, having shown, as we believe, that Cannon's supposed proof, by means of the heart reaction, of augmented epinephrin output through stimulation of the sciatic is illusory, we do not consider that the explanation of the mechanism of the reaction is our concern. We have simply made some suggestions. Nor do we judge it necessary to discuss in this paper his supposed proof of the augmenting action of asphyxia and of emotional excitement upon the output. Clearly all his conclusions upon this matter stand or fall together. It may be mentioned, however, that we have observed acceleration of the heart, induced by asphyxia, after removal of the adrenals.

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ESSENTIALS IN MEASURING EPINEPHRIN OUTPUT WITH FURTHER OBSERVATIONS ON ITS RELATION TO THE RATE OF THE DENERVATED HEART

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The measurement of the rate at which epinephrin is given off from the adrenals under any given conditions necessarily involves the measurement of a mass and a time, since that rate is the quantity of epinephrin liberated per unit of time. It seems absurd to set this down in so many words. And the absurdity would strike every reader if our subject were the rate of output of blood from the left ventricle, the rate of output of carbon dioxide from muscles, the rate of hydrolysis of starch by amylase or the rate of excretion of urea by the kidney. But curiously enough, in the case of epinephrin some writers seem to assume that the mathematical conditions which govern the measurement of every velocity can be circumvented. And quantitative conclusions as to the influence of this or that factor upon the rate of epinephrin output have been confidently deduced from reactions which are not even specific qualitative reactions for epinephrin (such as the paradoxical dilatation of the pupil or acceleration of the denervated heart) and from observations which did not comprise a single measurement of the quantity of epinephrin given off per unit of time. Indirect qualitative methods may have value in corroborating the results of direct quantitative methods, but in case of conflict the presumption must be in favor of the latter. We have recently (1) had occasion to call attention to the bearing of this generally accepted principle upon certain discrepancies in the literature in regard to the influence of asphyxia upon the rate of epinephrin output.

The straightforward way of measuring the mass of epinephrin given off per unit of time is to collect blood from the adrenal veins for a known time and to assay its concentration in epinephrin. Various methods of obtaining the blood may be adopted depending on the kind of animal

and other conditions of the experiments. Thus, in good-sized dogs a cannula may be inserted into the lumbo-adrenal vein on one side extraperitoneally, and blood collected from one adrenal. In cats the most convenient method is to form a cava pocket. This is a method which has been employed by a number of investigators, including Hoskins and McClure¹ (2) who, in addition to the cava and the veins entering it which Biedl (3) tied off, ligated the abdominal aorta. We also prefer to tie the abdominal aorta and renal arteries, in order to prevent too much blood from stagnating in the hind end of the animal and the kidneys when the cava and renal veins have been ligated. The arteries are tied a little time before the veins, so that as much blood as possible may drain out of the occluded parts. There is no obvious reason why the exclusion of the hind legs and kidneys should affect the output or the nervous mechanism governing it. Indeed, the better blood pressure maintained after ligation of these vessels ought to favor rather than to hinder any reflex nervous effects elicited, e.g., by stimulation of the central end of sensory nerves or any excitatory effects upon nerve centers exerted by such conditions as asphyxia, since the conductivity of the reflex arcs and the excitability of the nerve centers must be better maintained when the blood flow is increased. The ordinary spinal reflexes are well obtained in the parts whose circulation has not been interfered with, and indeed in the hind end for some time after ligation of the vessels (see, e.g., protocol of cat 436).

When Doctor Cannon (4) attributes to the peculiar method adopted by us for collecting the blood our failure to corroborate his results, he does not explain how our method is unfavorable to the eliciting of the reaction. He attempts to show that the output of epinephrin measured by us in adrenal vein blood, collected from the cava and assayed on rabbit intestine (and uterus) segments, is an artificial phenomenon. He says "the effect of opening the abdominal cavity, clamping off the inferior cava and repeatedly manipulating the abdominal contents,

¹ The epinephrin output determined by these observers in 5 dogs by assay of adrenal blood, collected in the manner described, on rabbit intestine segments agrees well with our results. If the 1:1,000,000 adrenalin solution, in terms of which the output is expressed, is assumed to contain about 75 per cent of base (it was probably not assayed) the average output would be 0.00019 mgm. per kgm. per minute. Our average for 17 dogs was 0.00022 mgm. per kgm. per minute. The average weight of our dogs was exactly half that of the dogs employed by Hoskins and McClure, and there is reason to believe that in the larger dogs the output of epinephrin per kilogram of body weight is somewhat less than in the smaller.

either in pressing blood out of the inferior cava or withdrawing it by syringe, must be examined." We do not press blood out of the inferior cava nor do we withdraw it by syringe, if that is a more serious insult to the animal, nor do we repeatedly manipulate the abdominal contents. We open the abdominal cavity, prepare the cava pocket and clamp it off when blood is to be collected from the cannula inserted in the lower end of the pocket. We have examined the influence of several of the factors mentioned upon the output and have not found any effect detectable by our methods (5), (6).

The alleged influence of opening the abdomen upon epinephrin secretion. Doctor Cannon does not state the result of his examination. All he says is that "fully twenty years ago Bayliss and Starling called attention to the profound effect which opening the abdominal cavity has on the intestines in causing them to become absolutely motionless. These well-established facts make an interesting commentary on the use of the cava pocket as a mode of obtaining evidence of normal or natural secretion." He proceeds, "There is no doubt that secretion from the adrenal medulla is subject to impulses delivered by the splanchnic nerves and there is no doubt that opening the abdominal cavity under anesthesia results in a discharge of impulses along these nerves. The adrenal glands, therefore, are continuously and abnormally stimulated if the abdomen is opened." If Bayliss and Starling had found that opening the abdomen caused the setting up of inhibitory impulses for the intestines, this would, of course, form a complication in the study of the intestinal movements under these conditions. Despite this, Cannon accepts their results as, so far as we are aware, everybody else does. But because he imagines that opening the abdomen sets up inhibitory impulses for the intestinal movements which, however, did not prevent Bayliss and Starling from making trustworthy observations, he concludes that our results on a totally different object, the adrenals, are completely vitiated by impulses set up in an altogether different group of nerve fibers, the epinephrin-secreting fibers, although neither he nor anybody else has ever shown that opening the abdomen influences them in the least. The only commentary we need make upon this is that Bayliss and Starling (7) do not call attention at all to the effect of opening the abdomen in causing the intestines to become absolutely motionless. On the contrary, they state that when they opened the abdomen in the warm-saline bath they found the intestines collapsed and absolutely motionless if the splanchnic nerves were intact. They drew the conclusion, not that

the opening of the abdomen caused the inhibitory impulses to the intestines to be set up but that these impulses were already descending the splanchnics before the abdomen was opened. Singularly enough, Cannon quotes their statement *verbatim*: "These facts suggest that in the *intact* animal, at any rate under the conditions of our experiment, tonic or reflex influences are continuously descending the splanchnic nerves, inhibiting the activity of the intestines." To be logical, then, he ought to conclude that since the impulses which pass to the intestines along the splanchnics are *not* called into existence by opening of the abdomen but are already present, impulses descending the splanchnics to the adrenals and sustaining a normal epinephrin output must also be present in the intact animal. If opening the abdomen so completely deranges the action of abdominal viscera, including the adrenals, a very large part of our supposed physiological knowledge must be wiped out. The truth is, of course, that we must neither assume in any particular case that opening of the abdomen is indifferent to the experiment nor that it absolutely contraindicates it, but must always test, as far as possible, whether and in what direction this operation, as well as the other experimental conditions, influence the result. We have pointed out elsewhere (22) that the relatively narrow range within which the output, (as measured by observers, including ourselves, who have employed methods correct in principle), varies with different anesthetics and different operations suggests strongly that what we term the normal or ordinary spontaneous output is not initiated or sustained by the trauma or the anesthesia.

To emphasize his criticism of our method of obtaining adrenal vein blood by opening the abdomen, Cannon states that the acceleration of the denervated heart, caused by stimulation of the central end of the sciatic, is hardly ever observed after opening the abdomen, although asphyxia still gives the reaction. He apparently considers this remark so important that he italicises the following sentence, "in the entire series of cases with opened abdomen there was only one in which sensory stimulation caused any effect ascribable to adrenal secretion." Since we have shown (8) that this heart reaction has no significance as an indication of augmented epinephrin output, the statement that acceleration on sciatic stimulation is rarely obtained after the abdomen has been opened would, even if true, have no bearing upon the question at issue. It is easy to demonstrate, however, that the reaction is readily obtained not only soon after the abdomen has been opened but long thereafter, and when the abdominal aorta, renal vessels and cava

have been tied or clipped off in making the cava pocket. Indeed, there was some indication that the reaction might be greater after clipping the abdominal aorta, presumably owing to the better blood flow in the nervous centers (see, e.g., protocol of cat 437 in the previous paper (8)). There is plenty of evidence in the protocols of that paper that opening of the abdomen does not interfere with the reaction in question, and this is further illustrated here in figures 1 to 4.



Fig. 1. Parts of blood pressure tracing from cat 175. *A*, before and *B*, a portion commencing 24 seconds after beginning sciatic stimulation; (abdomen opened, left splanchnic cut nearly 2 hours). In all figures line of zero pressure corresponds with time trace; time in seconds; numbers above time trace represent heart rate per minute.

The protocols of the experiments from which these tracings are taken have already been published (8). In figure 1 are reproduced two portions of the blood pressure tracing (from cat 175), one just before, *A*; and the other during stimulation of the sciatic, *B*. The pulse rate was increased by nearly 30 beats per minute. The abdomen had not only been opened almost 2 hours before, and the left splanchnic nerve cut, but the intestines had been purposely manipulated. The heart reaction had been obtained many times after opening the abdomen,

both by excitation of the sciatic and by excitation of the left splanchnic before this tracing was taken, but an excellent rise of pressure was still caused when either of these nerves was stimulated. Stimulation of the sciatic before the abdomen was opened and with both splanchnics intact gave an acceleration of 22 beats in one observation and 25 beats in another.

Figure 2 from another cat (177) shows an acceleration of almost 40 beats produced by sciatic stimulation after opening the abdomen and

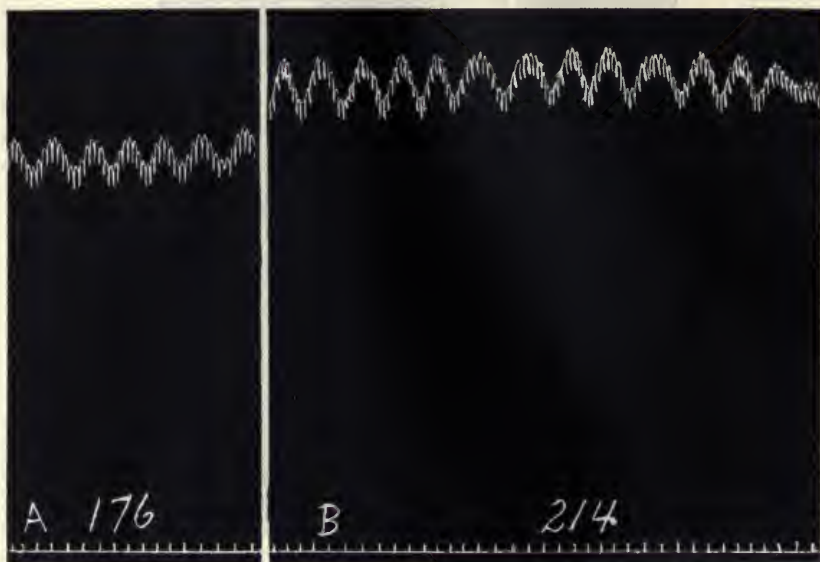


Fig. 2. Parts of blood pressure tracing from cat 177. *A*, before and *B*, a portion commencing 32 seconds after beginning of sciatic stimulation (abdomen open). Reduced one-nineteenth.

section of one splanchnic. Before the abdomen was opened, stimulation of the sciatic caused a maximum acceleration of 43 beats, with both splanchnics intact.

In figure 3 are reproduced portions of a curve (from cat 179) demonstrating a maximum acceleration of 34 beats, produced by sciatic stimulation before opening of the abdomen. One splanchnic had been previously cut extraperitoneally. After the abdomen was opened, a number of sciatic and splanchnic stimulations having been made in the meantime, stimulation of the sciatic caused an acceleration of 24

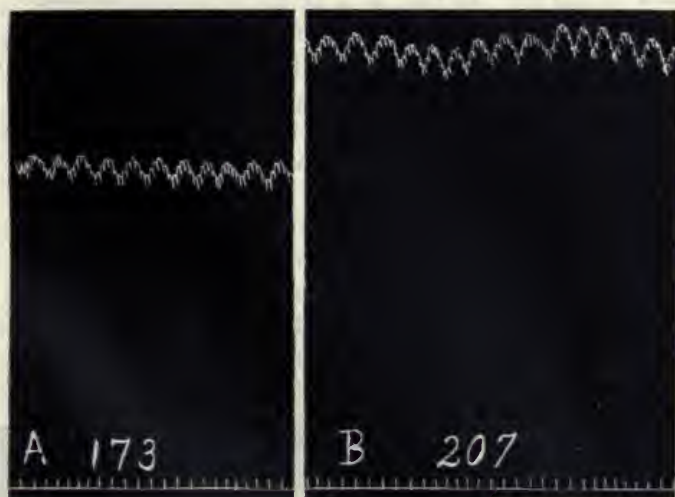


Fig. 3. Parts of blood pressure tracing from cat 179. *A*, before and *B*, a portion commencing 32 seconds after beginning of sciatic stimulation (left splanchnic cut).

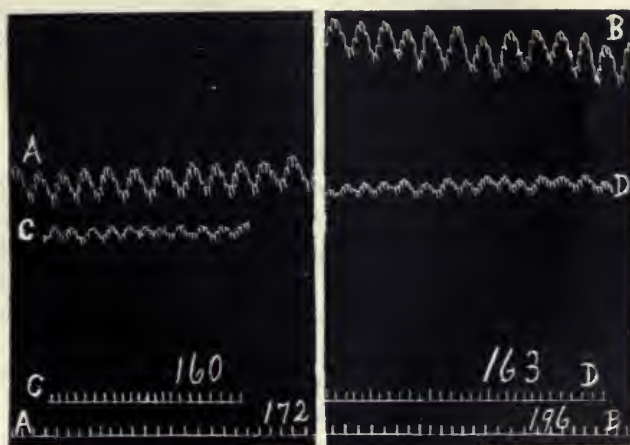


Fig. 4. Parts of blood pressure tracing from cat 179. *A*, before and *B*, a portion commencing 25 seconds after beginning of sciatic stimulation (left splanchnic cut and abdomen opened). *C*, before and *D*, a portion commencing 23 seconds after beginning of sciatic stimulation (both splanchnics cut).

beats (fig. 4, *A* and *B*). In this, as in all the other experiments, so long as sciatic stimulation gave a good rise of blood pressure, it gave a substantial acceleration of the heart. Later on in the experiment the second splanchnic was cut. The blood pressure fell to 44 mm. of mercury, and stimulation of the sciatic now caused little or no acceleration of the heart and only an insignificant rise of blood pressure (fig. 4, *C* and *D*).

If Cannon believes that the presence of the reaction indicates increased epinephrin output, it is incumbent upon him to explain why in adrenal blood collected at a time and under conditions when this reaction is well obtained, we are unable to detect, by a direct and sensitive method of assay (intestine segments), any sensible increase in the rate

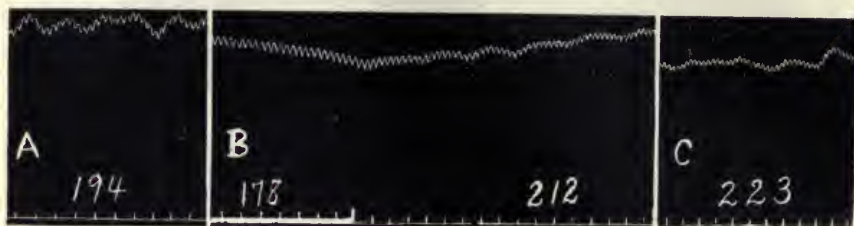


Fig. 5. Parts of blood pressure tracing from cat 436. *A*, before asphyxia for 60 seconds; *B*, 7 seconds before end and 16 seconds after end of asphyxia; *C*, a portion commencing 40 seconds after end of asphyxia (abdominal aorta and renal vessels tied). Reduced to four-fifths.

of output. In the case of asphyxia it is still more necessary that he should explain why we cannot detect such great increases in the epinephrin output as he assumes to occur. For he admits that the acceleration of the denervated heart associated with asphyxia is not abolished by opening the abdomen. We can confirm this entirely, and can add that the reaction can also be obtained after ligation of the abdominal aorta, renal arteries and veins and other vessels, as done in the formation of a cava pocket for the collection of adrenal vein blood. This is illustrated in figure 5 (from cat 436).

Condensed protocol. Cat 436; male; weight 2.26 kgm.

Left superior cervical ganglion excised 28 days previously. Under urethane (6 grams) cut vago-sympathetics, excised stellate ganglia, prepared central end of left sciatic for stimulation. The anesthesia was very deep and considerable depression of respiration and circulation was present from the start.

		<i>Rate</i>	<i>Pressure</i>
12:42 p.m.	Before sciatic stimulation.....	203	56
	6 seconds after beginning stimulation.....	206	62
	20 seconds after beginning stimulation.....	210	64
	40 seconds after beginning stimulation.....	204	64
12:52 p.m.	Before asphyxia for 45 seconds.....	135	26
	Just after beginning asphyxia.....	140	30
	10 seconds after beginning asphyxia.....	162	46
	26 seconds after beginning asphyxia.....	195	45
1:05 p.m.	Opened abdomen, tied abdominal aorta, renal arteries and veins		
1:10 p.m.	Before asphyxia for 45 seconds.....	184	49
	Just after beginning asphyxia.....	180	54
	15 seconds after beginning asphyxia.....	181	62
	Just after end of asphyxia.....	198	60
1:15 p.m.	Intravenous injection of 50 cc. Ringer's solution		
	Before Ringer injection.....	200	53
	After Ringer injection.....	204	80
1:35 p.m.	Before asphyxia for 60 seconds.....	194	66
	Just after beginning asphyxia.....	199	78
	30 seconds after beginning asphyxia.....	178	56
	Just after end of asphyxia.....	212	62
	20 seconds after end of asphyxia.....	219	53
	40 seconds after end of asphyxia.....	223	54
1:40 p.m.	Tied coeliac axis and superior mesenteric artery, clipped cava; hind leg and tail reflexes still present		
1:42 p.m.	Before asphyxia for 60 seconds.....	205	63
	During first 30 seconds of asphyxia.....	201	64
	Just after end of asphyxia.....	204	65
	15 seconds after end of asphyxia.....	218	60
	30 seconds after end of asphyxia.....	220	59
1:48 p.m.	Completed cava pocket		
1:49 p.m.	Before closure of pocket.....	208	56
	During closure of pocket.....	190	42
	Just after release of pocket blood.....	189	44
	15 seconds after release of pocket blood.....	215	58

In any case how does Cannon conceive asphyxia to act upon the epinephrin output? He says a short asphyxia acts through the nervous system, a longer period of asphyxia has a direct action. Now, why should the stimulating action of asphyxia on the part of the central nervous system which presides over the adrenal epinephrin secretion be abolished by tying the abdominal aorta? The effect of its ligation is to raise the blood pressure and therefore to keep up a more efficient circulation in the central nervous system and adrenals. How does this prevent the stimulation of the nerve centers by asphyxia? It does

not do so, and the reason we do not find an increased output of epinephrin with asphyxia must be that when tested by a quantitative method, the alleged increase is non-existent or too small to be detected.

The relation of the epinephrin output of the adrenals to the acceleration of the denervated heart associated with asphyxia. Cannon states that tying the lumbo-adrenal veins on both sides of the adrenals may not diminish in the least the acceleration of the heart produced by asphyxia of a given duration. This seems very good evidence that the reaction cannot be a quantitative reaction for epinephrin output. It is fully in accord with this conclusion that Cannon himself sometimes obtained a very large acceleration by asphyxia after section of both splanchnics. He endeavors to explain this by assuming that prolonged asphyxia stimulates the adrenal medullary cells directly. He brings forward no real evidence that this occurs. v. Anrep (9) states that after section of both splanchnics (in the dog) no reaction is elicited by asphyxia on the blood vessels of the denervated limb, and concludes that asphyxia has no direct action upon the adrenal medulla. Pearlman and Vincent (10), however, have not been able to obtain on the denervated limb any reaction in asphyxia which could be attributed to epinephrin. If asphyxia lasting for 90 seconds can stimulate the adrenal medulla directly so as to cause enough epinephrin to be given off to accelerate the heart by 68 beats a minute after section of the splanchnics, our failure to obtain evidence of this effect in adrenal vein blood directly assayed would be more puzzling than ever. For Cannon himself admits that the asphyxial acceleration with intact splanchnics is not interfered with by opening the abdomen, and how then should opening the abdomen abolish an action of asphyxia exerted directly upon the adrenal cells? Cannon's conclusion, that asphyxia stimulates directly the adrenal medulla, is simply a misinterpretation of his result that sometimes after division of both splanchnics asphyxia may cause a quickening of the denervated heart. Even if the heart reaction were specific for epinephrin, this would only indicate that some portion of the nerve supply of the adrenals might have been spared, and would not of itself prove a direct action. But as soon as it is shown that the reaction is not specific for epinephrin, and can be obtained after removal of the adrenals, the whole argument is seen to be baseless.

Cannon cites Czubalski (11) as having "adduced evidence that asphyxia if sufficiently prolonged may have a direct stimulating action on the adrenal medulla." The paper referred to is a preliminary note in which it is stated that even after section of the cord and bulb and also after division of the splanchnics

and vagi, a rise of blood pressure can be produced beginning usually at the end of the third or beginning of the fourth minute of asphyxia. The rise of blood pressure was very often accompanied by marked slowing of the heart. Czubalski attributes the increase of pressure to adrenalin liberated by the direct action of asphyxia on the adrenal medulla. The heart, of course, was isolated as regards its extrinsic innervation, and, according to Cannon, the characteristic response of the heart thus isolated to increased epinephrin output is an acceleration. It is curious that he should find confirmation of his result on the direct action of asphyxia on the adrenals in an effect upon the heart precisely the opposite of that which he associates with epinephrin. Czubalski's assertion that the general blood of a dog after asphyxia contains so much epinephrin that its presence is demonstrated by marked inhibition of a rabbit's intestine segment, when the defibrinated blood is made to displace "a nutritive solution," is of itself sufficient to show that his conclusions are not of any value. His statement that after removal of the adrenals asphyxia no longer causes a rise of blood pressure, is invalidated by the observations of Gley and Quinquaud (13).

In one of Cannon's experiments he obtained with an asphyxia lasting 90 seconds, which is what he considers a prolonged asphyxia, an acceleration of 28 beats before section of the splanchnics. After section of the splanchnics no acceleration was produced by an asphyxia of 60 seconds, but he does not state what acceleration, if any, was caused by an asphyxial period of the same duration as the control before splanchnotomy, so that there is no way of judging whether the section of the splanchnics in this case had anything to do with the negative result. If the asphyxial rise of pressure is a factor in the acceleration, it is easy to see that section of the splanchnics or injury to them in removal or ligation of the adrenals must interfere with the reaction, apart altogether from the interference with the epinephrin output. In the figure given by Cannon (fig. 2) there seems to have been no rise of pressure after removal of the adrenals till 45 seconds from the beginning of asphyxia, whereas before removal of the adrenals the curve shows a substantial rise of pressure before this, how much earlier it is impossible to say from the section of the curve reproduced.

It is unnecessary, however, to go into details of this kind. The best proof that Cannon's supposed demonstration of the augmenting effect of asphyxia upon the epinephrin output by means of the heart reaction is fallacious is that asphyxia can elicit marked acceleration in the absence of the adrenals.

Experiments showing that asphyxia can cause acceleration of the denervated heart in the absence of epinephrin from the adrenals. Several instances have been given in the previous paper (8). Thus in cat 438 the heart was beating at the rate of 175 a minute. The second adrenal had been removed 35 minutes previously by the abdominal route. Asphyxia was induced for 60 seconds and the heart rate rose to 225 beats a minute, the maximum acceleration being attained after resumption of respiration. The blood pressure was 64 mm. of mercury and

did not change much. Sciatic stimulation at this time caused little if any acceleration of the heart, although earlier in the experiment and after removal of the second adrenal it gave a good acceleration. In cat 440 after removal of the adrenals and exposure of the cervical cord, which was associated with a fall of blood pressure of 20 to 30 mm. of mercury, asphyxia for 45 seconds caused an acceleration of 13 beats during the asphyxial period and of 18 beats counting from the end of asphyxia, the blood pressure rising only slightly (from 84 to 88 mm. of mercury). Stimulation of the sciatic, which had caused good accelerations (as much as 42 beats a minute) after excision of the second adrenal, had at this time only a very slight effect upon the heart rate. With a subsequent asphyxia of 30 seconds, the heart rate diminished from 216 a minute before to 203 during the asphyxia, but recovered to the initial rate on stopping the asphyxia.

In a number of experiments the preliminary dissection for excluding the adrenals from the circulation was made by the abdominal route at the beginning of the experiment, and ligatures were placed but not tied. The abdomen was then closed. After one or more periods of asphyxia with the adrenals discharging, the ligatures were tied so as to occlude the adrenal arteries, the adrenal veins and the lumbar veins just before they cross the glands. The abdomen was closed and the effect of asphyxia on the heart rate was again observed. Finally the adrenals were excised by cutting between them and the ligatures. There was no bleeding and no further ligatures were required, showing that the glands had been completely excluded from the circulation. The protocol of cat 445 is given as an example.

Protocol. Cat 455; female; weight, 2.27 kgm. Under ether cut vago-sympathetics, excised stellate ganglia, opened abdomen and placed ligatures in position to occlude adrenal vessels but did not tie them.

		Rate	Pressure
12:52 p.m.	Before asphyxia (30 seconds)	284	130
	Just after end of asphyxia	313	118
	15 seconds after end of asphyxia	318	127
	30 seconds after end of asphyxia	304	126
12:55 p.m.	Before asphyxia (45 seconds)	268	125
	During first 15 seconds asphyxia	274	130
	During next 15 seconds asphyxia	278	114
	During next 15 seconds asphyxia	295	100
	Just after end of asphyxia	312	120
	20 seconds after end of asphyxia	323	132
	35 seconds after end of asphyxia	316	138

		Rate	Pressure
1:05 p.m.	Before asphyxia (60 seconds)	308	102
	During first 15 seconds asphyxia	303	105
	During next 15 seconds asphyxia	302	96
	Just after end of asphyxia	321	57
	15 seconds after end of asphyxia	335	72
	30 seconds after end of asphyxia	350	72
	50 seconds after end of asphyxia	334	76
1:12 p.m.	Tied off adrenal vessels		
1:14 p.m.	Before asphyxia (40 seconds)	283	102
	During first 12 seconds asphyxia	287	98
	Just after end of asphyxia	301	81
	15 seconds after end of asphyxia	321	95
	30 seconds after end of asphyxia	301	94
	45 seconds after end of asphyxia	298	94
1:18 p.m.	Before asphyxia (60 seconds)	283	95
	During last 15 seconds asphyxia	313	93
	Just after end of asphyxia	326	96
1:23 p.m.	Before asphyxia (60 seconds)	300	105
	During first 15 seconds asphyxia	300	109
	During next 15 seconds asphyxia	296	98
	During next 15 seconds asphyxia	308	98
	During next 15 seconds asphyxia	312	98
	Just after end of asphyxia	316	100
	15 seconds after end of asphyxia	310	100
	30 seconds after end of asphyxia	303	100
1:30 p.m.	Excised both adrenals; prepared central end of sciatic		
1:40 p.m.	Before asphyxia (35 seconds)	287	95
	During first 20 seconds asphyxia	285	98
	Just after end of asphyxia	280	64
	15 seconds after end of asphyxia	283	64
	30 seconds after end of asphyxia	284	64
	45 seconds after end of asphyxia	287	84
1:50 p.m.	Before sciatic stimulation (6 cm.)	264	67
	Just after end of stimulation	286	90
	15 seconds after end of stimulation	284	75
1:57 p.m.	Before asphyxia (60 seconds)	261	70
	During asphyxia	264	57
	Just after end of asphyxia	267	68
	20 seconds after end of asphyxia	269	69
	35 seconds after end of asphyxia	270	72
2:06 p.m.	Before asphyxia (45 seconds)	238	52
	During first 15 seconds asphyxia	238	57
	During next 15 seconds asphyxia	238	50
	Just after end of asphyxia	249	48
	15 seconds after end of asphyxia	247	44
	30 seconds after end of asphyxia	248	44
	50 seconds after end of asphyxia	247	60

		Rate	Pressure
2:14 p.m.	Before sciatic stimulation.....	238	49
	During first 15 seconds stimulation.....	239	63
	During next 10 seconds stimulation.....	242	64
	During next 10 seconds stimulation.....	246	65
	Just after end of stimulation.....	242	64
2:32 p.m.	Before asphyxia (45 seconds).....	244	62
	During first 25 seconds asphyxia.....	245	54
	Just after end of asphyxia.....	242	45
	20 seconds after end of asphyxia.....	240	49

It will be seen that ligation of the adrenals did not interfere with the acceleration of the denervated heart induced by asphyxia. The first asphyxia after ligation of the glands gave an acceleration of 38 beats, the second an acceleration of 43 beats per minute. The third asphyxia gave an acceleration of 16 beats, but the initial rate was already 300, which was 17 beats greater than before either of the two previous asphyxial periods. After actual removal of the adrenals in this experiment the maximum acceleration caused by asphyxia was slight (only 9 beats per minute). This, however, can have nothing to do with loss of epinephrin output since the glands had been entirely excluded from the circulation by the ligation.

We have had abundant evidence, if evidence were needed, that repeated asphyxiation is not an indifferent procedure for the heart. A heart which at first has responded by an acceleration during the asphyxial period, and usually a still greater acceleration immediately thereafter, may later on respond by some diminution in the pulse rate, becoming more manifest toward the end of the asphyxia, and succeeded by an increase on stopping the asphyxia, which may carry the rate beyond the initial value or not. In figure 2 of Cannon's paper (4) the heart rate toward the end of asphyxia (after the adrenals were tied off) was slower by 6 or 7 beats a minute than before the asphyxia. It then increased to the initial rate after respiration was resumed. As only a small portion of the curve is reproduced, it does not show whether later on the rate went beyond the initial value or not. When successive periods of asphyxia are superimposed upon a steadily declining blood pressure in the course of an experiment the loss or diminution of the heart reaction is apt to be particularly evident.

A good acceleration was still given when the sciatic was stimulated after excision of the adrenals, accompanied by a fair rise of pressure. It is scarcely necessary to point out that it is impossible to reconcile these results with Cannon's conclusion that the acceleration of the denervated heart is an index of the increased output of epinephrin from the adrenals caused by stimulation of the sciatic and by asphyxia. In cat 455 the heart rate after section of the vago-sympathetics and excision of the stellate ganglia was exceptionally great.

In the next experiment to be cited the heart rate was unusually low, not much more than half the rate in cat 455 and the blood pressure was also only about half.

Protocol. Cat 453; male; weight, 2.51 kgm. Under urethane (5 grams) cut vago-sympathetics, excised stellate ganglia, opened abdomen and placed ligatures in position to occlude adrenal vessels but did not tie them.

		Rate	Pressure
11:35 a.m.	Before asphyxia (45 seconds).....	150	66
	During first 20 seconds asphyxia.....	152	66
	During next 20 seconds asphyxia.....	152	72
	Just after end of asphyxia.....	153	76
	20 seconds after end of asphyxia.....	155	68
	35 seconds after end of asphyxia.....	156	71
11:38 a.m.	Before asphyxia (60 seconds).....	153	63
	During first 15 seconds asphyxia.....	151	63
	During next 20 seconds asphyxia.....	151	69
	During next 15 seconds asphyxia.....	157	85
	Just after end of asphyxia.....	166	77
	25 seconds after end of asphyxia.....	166	80
11:45 a.m.	Tied off adrenal vessels		
11:46 a.m.	Before asphyxia (60 seconds).....	143	57
	During first 20 seconds asphyxia.....	144	60
	During next 30 seconds asphyxia.....	147	82
	Just after end of asphyxia.....	151	66
	25 seconds after end of asphyxia.....	153	70
11:50 a.m.	Before asphyxia (85 seconds).....	143	59
	During first 30 seconds asphyxia.....	145	68
	During next 30 seconds asphyxia.....	146	76
	During next 20 seconds asphyxia.....	152	64
	Just after end of asphyxia.....	159	76
	25 seconds after end of asphyxia.....	160	74
	40 seconds after end of asphyxia.....	156	78
12:00 m.	Prepared central end of sciatic for stimulation		
12:02 p.m.	Before sciatic stimulation (5 cm.).....	153	63
	During sciatic stimulation.....	174	106
	Just after end of stimulation.....	181	75
	25 seconds after end of stimulation.....	171	75
12:10 p.m.	Before asphyxia (55 seconds).....	157	62
	During first 25 seconds asphyxia.....	158	64
	During next 30 seconds asphyxia.....	163	70
	Just after end of asphyxia.....	163	56
	20 seconds after end of asphyxia.....	170	63
12:15 p.m.	Excised both adrenals		
12:16 p.m.	Before sciatic stimulation (5 cm.).....	156	57
	During first 20 seconds stimulation.....	166	77
	During next 20 seconds stimulation.....	183	72
	Just after end of stimulation.....	171	53

		<i>Rate</i>	<i>Pressure</i>
12:22 p.m.	Before asphyxia (60 seconds).....	150	51
	During first 20 seconds asphyxia.....	150	54
	During next 20 seconds asphyxia.....	151	60
	During next 15 seconds asphyxia.....	157	42
	Just after end of asphyxia.....	153	41
	30 seconds after end of asphyxia.....	155	47
12:30 p.m.	Two more observations with asphyxia gave in the first a maximum acceleration of 6 beats, in the other none; sciatic stimulation caused no acceleration; blood pressure had fallen to 48 mm., and during asphyxia to 33 mm. of mercury.		

The greatest acceleration caused by asphyxia in cat 453 before ligation of the adrenals was 13 beats a minute; after ligation it was 17 beats a minute. After excision of the glands, already completely excluded from the circulation, the greatest acceleration obtained with asphyxia was 7 beats per minute, and at the end of the experiment practically no acceleration was caused. It is obvious that the absence of the reaction in the last observation could have nothing whatever to do with the absence of the epinephrin output of the adrenals. Sciatic stimulation before excision of the adrenals, but after their exclusion from the circulation, gave an acceleration of 28 beats, and after excision of the glands an acceleration of 27 beats per minute. How is it possible to maintain that these accelerations are due to increased output of epinephrin from the adrenals, or that the failure to obtain them is due to elimination of the epinephrin output? The experiment proves clearly that the acceleration caused by asphyxia cannot be due to the same factors as the acceleration caused by sciatic stimulation, since the latter is unchanged after excision of the adrenals. In other experiments an asphyxial acceleration has been obtained at a time when little or no acceleration was elicited by stimulation of the sciatic. Figure 6 gives portions of the blood pressure curve from cat 453, showing the effect of stimulating the sciatic before and after excision of the adrenals and the effect of asphyxia after the excision.

In figures 7 and 8 are reproduced portions of the tracings from cat 456, to show the effect of asphyxia before the adrenals were tied off (maximum acceleration 22 beats per minute), after the adrenals were tied off (maximum acceleration 25 beats per minute), after the adrenals were excised (maximum acceleration 13 beats a minute), and later on (no acceleration). As shown in the protocol, ligation of the adrenals did not alter the position of the maximum acceleration any more than its absolute amount. Counts of successive portions of the curves

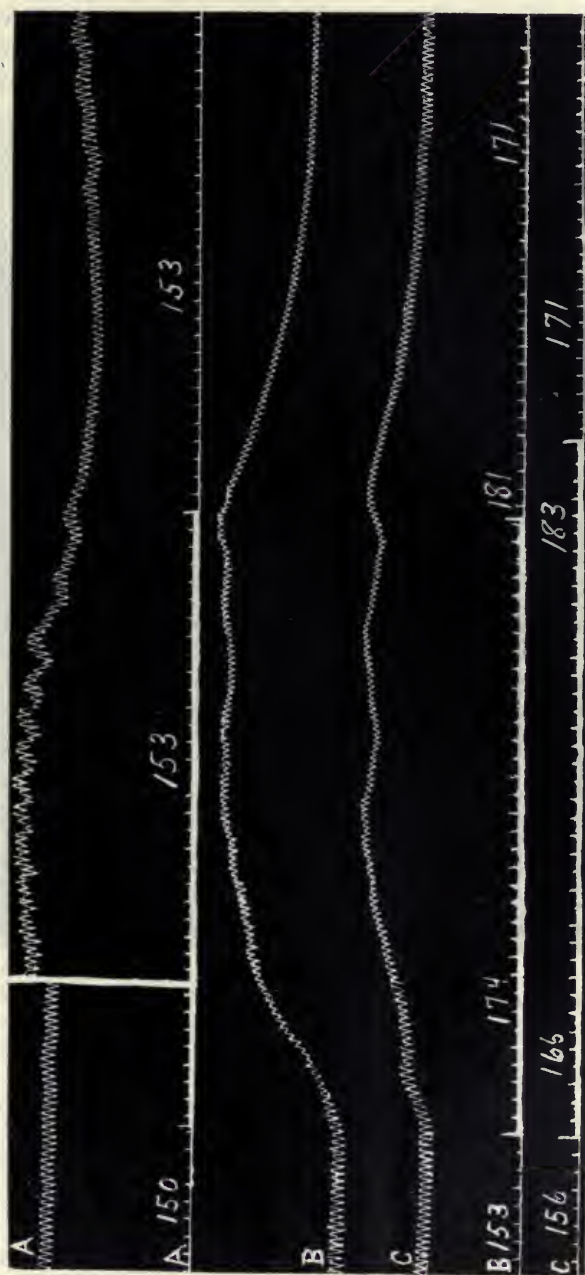


Fig. 6. Parts of blood pressure tracing from cat 453. A, asphyxia for 60 seconds, after excision of adrenals (26 seconds cut out of trace to save space); B, sciatic stimulation before, and C, after excision of adrenals. Reduced to three-fourths.

are given to establish this point, which is no more in harmony with the view that the acceleration is an index of epinephrin output than is the possibility of eliciting an undiminished acceleration after exclusion of the adrenals. Naturally, when, after ligation of the vessels, asphyxia was associated with a considerable fall of blood pressure no acceleration might be seen until respiration was resumed (Fig. 7). After re-

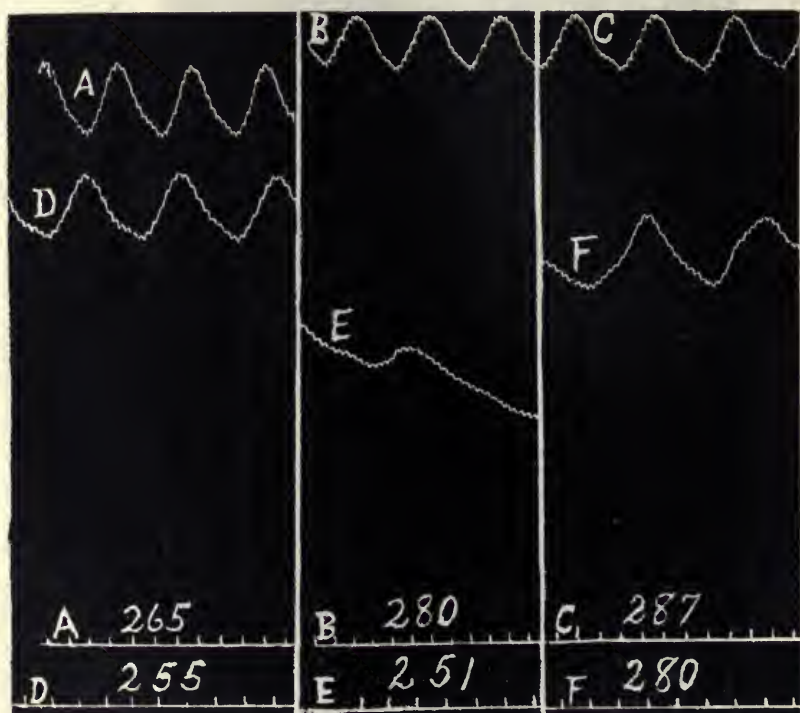


Fig. 7. Parts of blood pressure tracing from cat 456. *A*, before asphyxia, *B*, a portion just before end, and *C*, a portion commencing 18 seconds after end of asphyxia for 45 seconds, before tying adrenal vessels. *D*, before asphyxia, *E*, a portion just before end of asphyxia and *F*, a portion commencing 27 seconds after end of asphyxia for 45 seconds, after tying adrenal vessels.

peated asphyxiation the effect on the heart rate disappeared, although sciatic stimulation still caused a moderate acceleration (10 beats per minute). Again, it would be absurd to attribute the failure of the asphyxia reaction to elimination of the adrenals, since they were already

eliminated by the ligation and were cut out without bleeding, and since a fair acceleration was caused by asphyxia for some time after their removal.

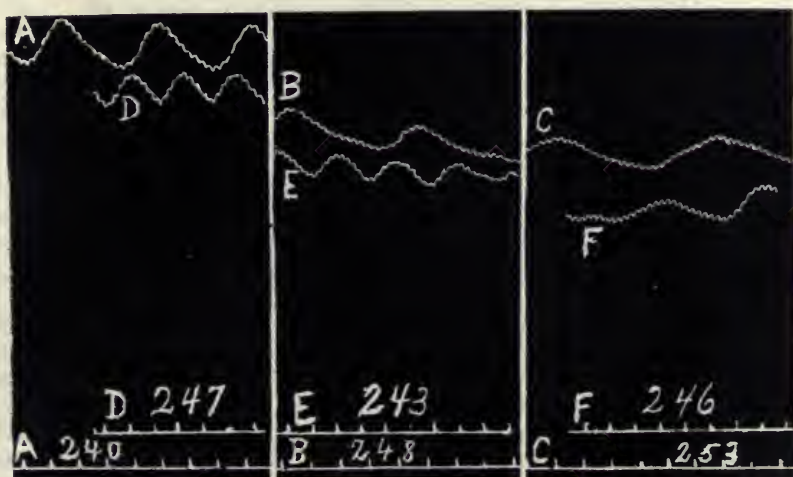


Fig. 8. Parts of blood pressure tracing from cat 456. *A*, before asphyxia, *B*, a portion just before end and *C*, a portion commencing 20 seconds after end of asphyxia for 45 seconds, after excision of adrenals. *D*, before asphyxia, *E*, a portion just before end and *F*, a portion commencing 21 seconds after end of asphyxia for 60 seconds, nearly half an hour after excision of adrenals.

Protocol. Cat 456; young female; weight, 1.55 kgm. Under urethane (3 grams) sectioned vago-sympathetics, excised stellate ganglia, opened abdomen and placed ligatures in position to occlude adrenal vessels but did not tie them.

		Rate	Pressure
11:53 a.m.	Before asphyxia (45 seconds).....	265	145
	During first 15 seconds asphyxia.....	270	156
	During next 30 seconds asphyxia.....	278	163
	Just after end of asphyxia.....	282	161
	15 seconds after end of asphyxia.....	286	159
	30 seconds after end of asphyxia.....	287	156
	50 seconds after end of asphyxia.....	283	150
11:58 a.m.	Tied-off adrenal vessels		
12:00 m.	Before asphyxia (45 seconds).....	255	130
	During first 15 seconds asphyxia.....	260	132
	During next 15 seconds asphyxia.....	259	114
	During next 15 seconds asphyxia.....	252	80
	Just after end of asphyxia.....	269	108
	15 seconds after end of asphyxia.....	278	114
	30 seconds after end of asphyxia.....	279	126
	45 seconds after end of asphyxia.....	280	126

		<i>Rate</i>	<i>Pressure</i>
12:10 p.m.	Excised both adrenals		
12:12 p.m.	Before asphyxia (45 seconds)	244	111
	During first 15 seconds asphyxia	245	116
	During next 20 seconds asphyxia	245	102
	During next 10 seconds asphyxia	247	94
	Just after end of asphyxia	251	94
	20 seconds after end of asphyxia	253	98
	40 seconds after end of asphyxia	253	98
12:15 p.m.	Before asphyxia (45 seconds)	240	110
	During first 20 seconds asphyxia	244	100
	During next 20 seconds asphyxia	249	88
	Just after end of asphyxia	248	80
	20 seconds after end of asphyxia	253	88
	40 seconds after end of asphyxia	252	92
12:18 p.m.	Prepared central end of sciatic for stimulation		
12:20 p.m.	Before sciatic stimulation (6 cm.)	240	88
	During sciatic stimulation	252	140
	Just after end of stimulation	251	108
12:23 p.m.	Before asphyxia (through 3 ft. tube) (60 seconds)	239	110
	During first 20 seconds asphyxia	238	103
	During next 20 seconds asphyxia	239	90
	During next 20 seconds asphyxia	242	80
	Just after end of asphyxia	241	67
	20 seconds after end of asphyxia	244	83
	45 seconds after end of asphyxia	247	92
12:30 p.m.	Before asphyxia (105 seconds) interrupted by 5 seconds respiration each half minute	240	96
	During first 20 seconds asphyxia	242	90
	During next 20 seconds asphyxia	243	85
	During next 20 seconds asphyxia	245	86
	During next 15 seconds asphyxia	244	80
	During next 20 seconds asphyxia	248	82
	Just after end of asphyxia	246	80
12:34 p.m.	Before sciatic stimulation (5 cm.)	242	88
	During first 15 seconds stimulation	247	106
	During next 20 seconds stimulation	252	120
	Just after end of stimulation	250	102
	20 seconds after end of stimulation	247	90
12:37 p.m.	Before asphyxia (60 seconds)	247	89
	During first 20 seconds asphyxia	245	76
	Just after end of asphyxia	243	57
	20 seconds after end of asphyxia	243	68
	45 seconds after end of asphyxia	246	72
12:42 p.m.	Before sciatic stimulation (6 cm.)	242	78
	During first 15 seconds stimulation	245	100
	During next 20 seconds stimulation	252	117
	Just after end of stimulation	252	99
	20 seconds after end of stimulation	250	86

		Rate	Pressure
12:50 p.m.	Before asphyxia (80 seconds).....	250	72
	During first 20 seconds asphyxia.....	251	82
	During next 20 seconds asphyxia.....	251	66
	During next 20 seconds asphyxia.....	252	64
	Just after end of asphyxia.....	248	58

In one cat (452) we obtained practically no acceleration with asphyxia (maximum increase in heart rate 5 beats per minute) after removal of the adrenals, although before removal a maximum acceleration of 42 beats had been got in one observation. In the very first observation, however, with a somewhat shorter asphyxial period the acceleration was very slight (6 beats per minute). The blood pressure was exceedingly high after denervation of the heart (214 mm. of mercury). After removal of the adrenals by the abdominal route it sank to 114 mm. of mercury, and the pulse rate declined nearly 40 beats a minute.

Condensed protocol. Cat 452; old male; weight, 4.17 kgm. Under urethane (5.5 grams) cut vago-sympathetics and excised stellate ganglia

		Rate	Pressure
11:30 a.m.	Before asphyxia (30 seconds).....	266	214
	During first 15 seconds asphyxia.....	260	214
	During next 15 seconds asphyxia.....	253	214
	Just after end of asphyxia.....	270	207
	15 seconds after end of asphyxia.....	272	215
	35 seconds after end of asphyxia.....	265	206
11:35 a.m.	Before asphyxia (45 seconds).....	258	190
	During first 20 seconds asphyxia.....	258	207
	During next 15 seconds asphyxia.....	256	220
	During next 10 seconds asphyxia.....	271	205
	Just after end of asphyxia.....	295	196
	20 seconds after end of asphyxia.....	300	203
	40 seconds after end of asphyxia.....	284	200
12:07 p.m.	Excised both adrenals (extraperitoneally)		
12:08 p.m.	Before asphyxia (45 seconds).....	222	114
	During first 20 seconds asphyxia.....	222	118
	During next 25 seconds asphyxia.....	219	121
	Just after end of asphyxia.....	222	102
	20 seconds after end of asphyxia.....	224	115
	40 seconds after end of asphyxia.....	223	115
12:14 p.m.	Before asphyxia (30 seconds).....	218	110
	During first 15 seconds asphyxia.....	223	118
	During next 15 seconds asphyxia.....	223	127
	Just after end of asphyxia.....	220	110
	20 seconds after end of asphyxia.....	220	120
	35 seconds after end of asphyxia.....	223	120

12:18 to 12:35 p.m. Three more observations with asphyxia (60 to 90 seconds) gave a small drop in the rate during the asphyxia; the blood pressure had fallen to 84 at the end of the experiment.

We do not think anybody who has studied our previous results will attribute the failure of the reaction in this experiment, after adrenalectomy, to elimination of the epinephrin output of the adrenals. But if by any chance a reader chooses to conclude, as apparently Doctor Cannon would do, that the drop of 36 beats a minute in the rate after removal of the glands is due to the absence of the epinephrin previously being given off from the adrenals (average 0.0002 mgm. per kgm. per minute in etherized cats) he will have difficulty in seeing why an increase in the output to many times this amount, with asphyxia, should be necessary to cause an acceleration of 42 beats a minute before the adrenalectomy, and why it should be impossible that a redistribution of the epinephrin without any increase in the rate of output might be responsible for the acceleration, even if asphyxia produces no change in the susceptibility of the heart to the action of epinephrin. The only possible conclusion, however, from our experiments is that whether the epinephrin takes any share in the acceleration associated with asphyxia or not, the reaction cannot be due solely to the adrenal epinephrin, and there is no evidence that epinephrin is concerned in it at all.

As has been pointed out in the previous paper (8), our proof that the increase in the heart rate caused by stimulation of the sciatic cannot be an index of an increased epinephrin output, does not entail the obligation to explain the acceleration, which is probably a complicated reaction. Still less do we feel bound to explain the asphyxial acceleration, which is possibly more complicated still. It is for Doctor Cannon, who brings forward the reaction as a quantitative test for changes in the epinephrin output, to exclude the other possible factors. We have suggested, however, (12) one possibility which must be controlled in so far as epinephrin may be a factor at all, namely, that asphyxia might render the heart more sensitive to such amounts of epinephrin as were being given off before the asphyxia was induced. Since we have shown that asphyxia in the absence of the adrenals can cause acceleration, it seems obvious that the accelerating action of a given dose of epinephrin might be reinforced by asphyxia. In that paper (12) we stated that it is not permissible to make quantitative comparisons on the effect of such a condition as asphyxia, using a test object like the heart well supplied with oxygen in one observation but asphyxiated in the comparison observation, without controlling any possible effect produced by the asphyxia upon the reactivity of the test object itself. There was every reason to point this out, as this factor is habitually

neglected by certain writers, who have worked with test objects *in situ*, although it must be taken account of before their results can be used at all.

Cannon now endeavors to control the possibility that asphyxia might alter the reactivity of the heart to one and the same dose of epinephrin, so that a reaction might be obtained simulating an increased output when the output was really unchanged. Although the question loses much of its interest, as a question in the technique of estimating epinephrin output, when it is known that the heart reaction relied upon by Cannon to demonstrate increased output is readily obtained in the absence of the adrenals, it may nevertheless be noted how Cannon takes account of the factor in question. He reproduces a curve taken while adrenalin, to the amount of 0.08 mgm. per minute, was being continuously injected into the veins of a cat. This is, for an average cat under ether, 200 times the mean rate of output of epinephrin under the conditions of our experiments, which according to Cannon may artificially increase the output to an "unsurpassable limit." The concentration of epinephrin in the blood passing through the coronary circulation must, therefore, have been much greater than could probably ever be sustained by the output from the adrenals, even if, according to Cannon, this were greatly increased by excitation of sensory nerves or asphyxia. The increase in the heart rate, however, was only 40 beats (from 132 to 172 per minute), an acceleration of the same order of magnitude as may be given by sciatic stimulation or asphyxia alone, without any artificial injection of adrenalin. Now, since the amount of adrenalin injected is, according to Cannon's own data, far greater than that liberated from the adrenals by asphyxia, it must be assumed that the acceleration was maximal before asphyxia was allowed to act. How can a possible change in the reactivity of the heart to epinephrin under the influence of asphyxia be demonstrated if the heart is already making its maximum effort in response to an enormous dose of injected adrenalin? The slowing of the heart, with the drop of pressure as the asphyxia continued, does not indicate that asphyxia of moderate duration renders the heart less sensitive to epinephrin, but is a very common phenomenon in asphyxia when no adrenalin has been injected, and when none can be coming from the adrenals. Epinephrin liberated at the ordinary rate may possibly enable the heart to resist better, and for a longer time, the depressing influence of asphyxia, and to respond better to the factors responsible for the acceleration either during the asphyxial period, or when respiration is resumed.

Another factor in the asphyxial acceleration, if epinephrin takes any share in it, may be, as already pointed out in the case of the acceleration caused by stimulation of sensory nerves (8), the redistribution of the blood associated with the vascular changes, which may result in a greater quantity of epinephrin per unit of time or a greater concentration of it being supplied to the coronary circulation, without any increase having occurred in the rate of output from the adrenals.

In another paper (12) we cited some evidence that certain reactions may be produced by epinephrin in this way, for instance, an experiment of v. Anrep (9) in which he shows that "if one splanchnic nerve is intact while the suprarenal on the other side is extirpated, stimulation of the splanchnic nerve on the side of the extirpated suprarenal may still cause constriction of a denervated limb. Only after the other splanchnic nerve is cut does the constriction disappear and the limb react passively to the change of blood pressure." His interpretation of this result is that "this is due to the fact that stimulation even of the peripheral end of the splanchnic excites a certain number of afferent nerves, so that there may be a reflex excitation of the suprarenal of the other side through the intact splanchnic nerve." There is no evidence that stimulation of the peripheral end of the splanchnic nerve can affect reflexly the rate of epinephrin secretion from the other adrenal and excellent evidence against it. The true explanation, we believe, is that so far as epinephrin is a factor in v. Anrep's reaction, the constriction seen under the circumstances described is due to the shunting through the denervated limb of more of the epinephrin being given off at the ordinary rate from the other adrenal. Cannon has suggested that in the cat (efferent) adrenin secretory fibers may occasionally pass from one splanchnic to the opposite adrenal, but he produces no proof of such a crossing. We have never seen evidence of any increase in the output of one adrenal when the opposite splanchnic was stimulated, or any evidence of diminished output when the opposite splanchnic was cut. Elliott's results on the protection of the epinephrin store from depletion in the adrenal whose nerves are cut, as compared with its fellow, could hardly have been what they were if both glands were innervated from each splanchnic. In the dog, v. Anrep could scarcely have failed to obtain the limb reaction in the experiment mentioned, after section of the opposite splanchnic, if there was a crossing of efferent fibers from the stimulated splanchnic to the other gland.

Redistribution of the blood owing to vasomotor changes is certainly not the only factor, if it is a factor, in the asphyxial acceleration, and in most of our experiments the rise of pressure produced by asphyxia after denervation of the heart was not great. Good accelerations were obtained with little or no increase of pressure, and the maximum acceleration was generally found when the pressure had fallen again, or, although rising after resumption of respiration, was still below the initial value. There was no obvious difference in this regard whether the adrenals had been excluded or not. Accordingly, such an experiment as Cannon illustrates in figure 7 of his paper (4) has no bearing on the question whether acceleration of the heart caused by asphyxia is due to increased epinephrin output. He denervated the heart, tied the limb and carotid arteries and severed the mesenteric nerves, and still got an increase in heart rate from 180 to 212 beats per minute after asphyxia, "with no previous noteworthy change in blood pressure." The pressure rose about 25 mm. of mercury after stopping the asphyxia,

and it was at this time that the maximum acceleration was counted. But how can this be a proof that asphyxia augments the output of epinephrin since equally large accelerations have been obtained by us in the absence of any epinephrin discharge?

As regards Cannon's method of demonstrating adrenal secretion by the rise of pressure caused by asphyxia after tying the carotid and limb arteries and "denervating the splanchnic area," it may be noted that the curve reproduced in figure 3 of his paper shows a slight elevation (less than 10 mm. of mercury) after the first minute of asphyxia, and a very large rise (at least 110 mm. of mercury) after resumption of respiration at the end of two minutes of asphyxia. He attributes both rises to the action of an increased output of epinephrin, but unfortunately does not state how much epinephrin would have been required to raise the pressure by 110 mm. of mercury. If such an enormous outpouring of epinephrin as would seem necessary to cause an effect of this kind were actually induced by asphyxia, it is impossible to see why we always missed it in collecting adrenal vein blood. Our operation was assuredly no more "severe" than Cannon's, the abdomen was opened by him also, and he states that the method yielded constant results so far as the belated influence of asphyxia was concerned, although "it was commonly disappointing as a means of demonstrating the early influence of asphyxia." If asphyxia can cause the liberation of so much epinephrin that a rise of pressure of this order of magnitude can be produced, it is also unintelligible that the careful experiments of Gley and Quinquaud (13) should not have revealed a striking difference in the effects of asphyxia on the blood pressure curve before and after eliminating the epinephrin output. They found that the curves were practically identical. Cannon's statement that after tying off the adrenals the rises of pressure did not occur, may mean nothing more than that the considerably lower blood pressure (during asphyxia it fell to little over 25 mm. of mercury) had so injured the heart that it could not respond to the resumption of respiration by such an increased action as, in the restricted circulation, would cause a marked rise of blood pressure, whereas, with the better circulation before exclusion of the adrenals it could do so.

These suggestions as to possible ways in which epinephrin may be a factor in the asphyxial acceleration of the denervated heart, without any increase in its rate of output having occurred, are not intended to imply that there is evidence that epinephrin takes any share in the acceleration. Our experiments show conclusively that it cannot be

the sole factor, if it is a factor at all, since marked accelerations can be obtained after exclusion or removal of the adrenals. As to the mechanism of the acceleration, we do not propose to enter further into a discussion of this reaction, in which direct effects of asphyxia upon the heart and indirect effects produced through the changed quality and quantity of the blood supplied to it may be intermingled. Our purpose was accomplished as soon as the statement that the reaction can be used to demonstrate an increased output of epinephrin during asphyxia was shown to be without foundation. Still less than in the case of the acceleration caused by sciatic stimulation can we imagine how such a reaction could be employed to measure quantitatively the rate at which epinephrin is given off by the adrenals.

Knowlton and Starling, working with the heart-lung preparation, have shown that the rate of the isolated mammalian heart is very sensitive to changes of temperature, although not altered by marked variations of arterial or venous pressure. But we have not included change of temperature of the blood coming to the heart during asphyxia or sensory stimulation, or change of temperature of the heart directly produced in asphyxia by stoppage of the heat loss through the respiratory tract, among the factors possibly concerned in the reaction because there is no evidence that under our experimental conditions this could have had any appreciable influence.

Cannon states that "the completely denervated heart can be used as an indicator of adrenal secretion in testing the influence of emotional excitement quite as well as in testing the influence of sensory stimulation and asphyxia." We have shown that it cannot be used at all in the case of sensory stimulation or asphyxia, and as, according to Cannon, it has the same value as a test for the influence of emotional excitement, we do not judge it necessary to discuss the matter here at any length.

Experiments on emotional excitement are necessarily less satisfactory than on sciatic stimulation or asphyxia because the animal must not be anesthetized. As a matter of fact, the evidence adduced by Cannon is extremely scanty and, in our opinion, will not bear examination. In one case, for instance, after removal of the left adrenal gland and section of the right splanchnic nerve in the thorax on the previous day an increase of 42 beats a minute was observed under excitement. In another case after a similar operation an increase of approximately 28 beats a minute was seen during excitement. These accelerations are of the same order of magnitude as those seen with both adrenals and their

nervous supply intact. How is it possible to consider a reaction as a quantitative test of the rate of epinephrin output which is given as well with one adrenal removed and the innervation of the other crippled as when both are normally discharging epinephrin? These animals had had many hours to recover from the effects of the anesthetic and operation and, therefore, gave a good heart reaction with excitement, a reaction which probably had little if anything to do with the assuredly diminished output of epinephrin from the remaining adrenal. That after removal of the remaining adrenal the reaction was not obtained when the animal was allowed to recover from etherization, is no proof that the failure of the reaction was due to the suppression of the residual epinephrin output of the gland, which was already largely denervated. The negative result is far more likely to have been due to the general condition of the animal just recovering from a second etherization and a second operation.

The only other experiment mentioned by Cannon is one in which excitement increased the heart rate from 217 to 255 beats per minute with the adrenals intact. Two days after this observation the adrenals were removed, of course under anesthesia, and about 5 hours thereafter excitement caused the rate to increase from 217 to 221 beats a minute. The conclusion is drawn that the difference in the result before and after the adrenalectomy is due to the loss of the epinephrin secretion. The possibility that the general condition of the animal after the adrenalectomy might be less favorable to eliciting a large acceleration is not considered. It must again be pointed out, as in the case of sciatic stimulation and asphyxia, that even if it had been proved that the failure of the reaction after removal of the adrenals was due to the absence of the adrenal epinephrin output and to this alone, the conclusion would not be justified that emotional excitement increases the rate at which epinephrin is liberated into the blood, until it was shown that the changes in the amount and concentration of the epinephrin passing through the coronary system, occasioned by the vascular effects of the excitement, were insufficient to account for the observed accelerations. We should have considered it a mere accident that the heart rate was the same (217 beats a minute) after removal of the adrenals as two days before. Cannon, however, believes that this "is an indication, and the first reliable one, that under quiet, peaceful conditions there is no adrenal secretion or a secretion so slight as not to affect the denervated heart, an extremely sensitive indicator." He argues that if there was a secretion of the magnitude observed by us there ought to have been a drop in the pulse rate when this secretion was eliminated. Doctor Cannon has not mentioned what the pulse rate was just before the adrenalectomy, so we do not know whether there was a decline in the rate or not after removal of the glands. We have, however, presented evidence both in the preceding paper (8) and in this, that other factors than the loss of epinephrin are concerned in the diminution of the pulse rate which usually follows removal of the adrenals and that sometimes that operation, or an equivalent interference with the epinephrin

output may not be followed by any diminution in the rate, while after exclusion of the adrenals a marked diminution may be caused by operative and other procedures associated with a fall of blood pressure.

The catheter method. Cannon sums up his work with the heart reaction by the declaration that "the results obtained with the isolated heart used as an indicator of adrenal secretion confirm in every respect the results obtained eight years ago by the catheter method." We have shown what kind of confirmation the isolated heart used as an indicator of adrenal secretion can lend to any other method, it could only render it suspect. Since, however, despite their confirmation by the isolated heart reaction, the results obtained by Cannon with the catheter method might be correct, we shall take this occasion to examine them on their merits in somewhat greater detail than has hitherto been done. This series of papers by Cannon and his co-workers deals with the influence of emotional excitement, asphyxia, stimulation of sensory nerves and some drugs upon the rate of epinephrin output. He collected blood from the inferior vena cava above the level of the adrenal veins by means of a catheter pushed up through the femoral vein. According to him, blood obtained from this region in cats in the absence of excitement, asphyxia, etc., does not cause inhibition of cat's intestine strips or rabbit's intestine segments, whereas blood obtained during or after the action of these factors causes marked inhibition, due to the outpouring of epinephrin. After emotional excitement lasting for 10 minutes or more, so much epinephrin has been or is being liberated that it can be detected in the general venous blood (femoral vein), after passing not only through the lungs but through the systemic capillaries. It may be remarked that if this is correct, it ought not infrequently to be possible to detect epinephrin in blood collected from patients or animals by puncture of a vein. For much excitement may attend even so small an operation. But no satisfactory evidence has ever been brought forward that detectable concentrations of epinephrin exist in the general venous blood. To account for the extraordinary output of epinephrin which would be necessary in order that it should be clearly detected in femoral vein blood, Cannon suggests that adrenalin liberated into the blood stimulates the adrenals to further increase the output. "Thus the more marked effect as time passes (see fig. 3) may be due not only to further excitement, but in part to an autogenous continuance of adrenal secretion. Thus also the persistence of the emotional state after the exciting object has disappeared can be explained. Indeed it was the lasting effect of excitement on digestive processes

which suggested this investigation." In support of this idea he appeals to a statement of Elliott's (14) that adrenalin itself causes depletion of the epinephrin store of the adrenals. Elliott himself later withdrew this statement (15), which in any case would not prove that adrenalin increased the output of epinephrin.

Cannon complains that "authors have written as if Cannon had been attempting to support the idea that emotional experiences were dependent upon circulating adrenin. Thus Stewart and Rogoff report, as if the matter had been questioned, that all signs of fright can be elicited by administering morphine to a cat with one adrenal removed and the other denervated." This subject is not referred to at all in the paper quoted by Cannon. In another paper (26), we are discussing the depletion of the epinephrin store produced by morphine, which Elliott (15) suggests is directly due to the fright caused by the drug. In this connection we say "That signs which might be interpreted as those of fright are present in cats under morphine is, of course, not doubtful. Whether this interpretation is correct might be difficult to decide, and does not concern us here. It is, however, of interest to note that epinephrin seems to have nothing to do with those signs. The signs of morphine fright can all be elicited by administering morphine to a cat in which one adrenal has been removed and the splanchnic supply of the other cut and in which accordingly no liberation of epinephrin through the splanchnics takes place." How can this be spoken of as "writing as if Cannon had been attempting," etc.? As a matter of fact, in that paper we were not discussing Cannon's psychological theories at all. We do not see that there was any impropriety in publishing observations showing that signs, which might be interpreted as those of fright, can be elicited by morphine in cats whose epinephrin output has been abolished or markedly diminished, even if we had been aware, which of course we could not be, that this detail in the pharmacology of the drug would seem self-evident to Cannon.

Cannon complains further that "Rogoff points out that the secretion of sufficient adrenin to produce symptoms of fright would be impossible, again as if any claim had been made that these symptoms were due to secreted adrenin." What Rogoff did say was: "In some of the cats (after removal of one adrenal and denervation of the other) it was found that the liberation of epinephrin from the adrenals was so interfered with by this operation that there could not have been one-thousandth of the normal liberation, if any epinephrin was being given off from the glands. These animals, nevertheless, responded readily to fright and other emotional disturbances with the usual symptoms of sympathetic excitation in the same manner as normal cats. Certainly, an outburst, through nervous influence, of epinephrin in such quantities as would be necessary to produce these symptoms would be impossible in these animals." Again, we fail to see the impropriety of quoting such a result, so long as it is true. We are glad that Doctor Cannon finds this result so obvious as not to be worth while mentioning. For it must then be obvious to him also that in these animals "the persistence of the emotional state after the exciting object has disappeared" cannot be explained as due to "continuance of adrenal secretion," whether "autogenous" or not.

Cannon seems to criticise our use of the term "denervated eye" for the eye after removal of the corresponding superior cervical ganglion, and says that he and de la Paz "tried the denervated eye method of testing for adrenal secretion but could not persuade themselves that an eye still innervated by the third cranial nerve was really denervated." It is surely unnecessary to state that when we use the term "denervated eye" in this sense we do so for convenience, following the practice of other writers, for instance, Elliott (15). We frequently speak of "the (denervated) eye reactions of Meltzer," or simply "the eye reactions," or of "the iris sensitized by previous removal of the superior cervical ganglion." Cannon himself uses the terms "excited" blood and "quiet" blood for convenience, and quite properly, although he will not maintain that they are literally correct. We did not imagine that any reader would suppose that we were not aware that the oculomotor nerve supplies the eye. We agree with Cannon that "the prompt dilatation of the pupil in a paroxysm of rage," in cats whose epinephrin output has been interfered with by removal of one adrenal and section of the nerves of the other has nothing to do with epinephrin, but is due to inhibition of the pupillo-constrictor mechanism. We have attributed the dilatation caused by stimulation of sensory nerves, including the paradoxical reaction, which can be obtained after removal of the adrenals (8), to the same mechanism. Long ago, it was stated by one of us (27) that sexual excitation caused very marked dilatation of the pupils, in dogs of both sexes, after division of both vago-sympathetic nerves (without removal of the superior cervical ganglion) by central inhibition of the constrictor pupillae through the oculomotor.

Apparently it is necessary to point out again, as has been done repeatedly before (1), (12), that when we employ the eye reactions to test for epinephrin, we eliminate factors which can affect the eye through the nervous system, by collecting the adrenal blood in a cava pocket and only releasing it, as far as possible, after these factors have ceased to act.

Cannon, on the basis of his experiments with the catheter method, concluded that emotional excitement, asphyxia and sensory stimulation increase the rate of output of epinephrin. Nicotine does the same, whereas urethane has no effect. Our own experiments with asphyxia and sensory stimulation, made by methods which permitted the rate of output of epinephrin to be estimated, did not support Cannon's conclusion, yielding no evidence of sensibly increased output. Such negative results must always yield to positive results obtained by a better method, and this has been emphasized by us. Cannon's catheter method, however, far from being a better method than those employed by us, is not a method by which the rate of output of epinephrin can be estimated at all. As a matter of fact, his papers do not contain a single estimate of the output of epinephrin before, during or after the action of the factors which he is studying. And it is clearly impossible that they should contain such data. The most that the method could yield would be the epinephrin concentration in the cava blood above the

adrenal level. Without knowing the amount of blood with this epinephrin concentration passing the point of collection per unit of time, the output of epinephrin could not be calculated. But even the epinephrin concentration was not determined by Cannon. He gives not one estimate of the concentration, or any information as to the minimum concentration which his strips or segments could have detected. In the absence of information of this kind it is impossible to deduce any conclusion from the few curves reproduced. What, for instance, was the concentration of epinephrin in the femoral vein blood after emotional excitement? If we knew whether it was 1:1,000,000 or 1:10,000,000 we might be able to check the probability of the statement by calculating the concentration which must have existed in the blood of the adrenal veins, and seeing whether anyone has ever observed so high a concentration. If the minimum concentration clearly detectable by the segments and the concentration actually found in the cava blood during asphyxia, etc., had been determined, there would be some possibility of deciding whether the observed changes in concentration could be due to alterations in the rate of blood flow in the cava without any change having occurred in the rate of output of epinephrin.

We have suggested that vasoconstriction, especially in the splanchnic area, which may be assumed to accompany the rise of blood pressure associated with stimulation of the central end of the sciatic or with asphyxia, will cause slowing of the blood flow in the inferior cava. Doctor Cannon seeks to invalidate this suggestion by the statement that an increased arterial blood pressure causes an increased blood flow through the *adrenals*. Had this argument not been repeated and emphasized in his paper we should have considered it a slip. Certainly the adrenal flow increases when the arterial pressure rises. But as the blood from the adrenals is only a small fraction (perhaps $\frac{1}{100}$ to $\frac{1}{200}$) of the blood passing along the inferior cava this, of course, has no sensible influence upon the rate of cava flow. Cannon further argues that when the arterial pressure is raised by splanchnic vasoconstriction the blood flow in the inferior cava may be increased. We had supposed it was universally admitted that vasoconstriction renders the passage of the blood through a vascular area more difficult, and that the arterial blood pressure rises for this very reason when an important area, like the splanchnic area, is constricted. We are not concerned here with compensatory reactions which may occur in other areas, leading, for example, as Edwards (16) has shown, to increased flow in the superior cava. It is precisely because less blood is passing through the vasoconstricted areas that more blood can pass through areas which are not constricted.

Vasomotor effects are not the only factors which may influence the rate of the blood flow in the inferior vena cava under the conditions of Cannon's experiments. The respiratory movements and, therefore, the intrathoracic pressure are affected by sensory stimulation, emotional disturbance and very grossly by

asphyxia. It is not possible to produce such changes without markedly affecting the pressure in the great veins. The region of the cava from which a sample of blood is assumed to be drawn off through the catheter is right up against the diaphragm. How can the control sample be obtained under the same conditions, except for the hypothetical stimulation of the adrenal secretory mechanism, as a sample taken when the animal is gasping in asphyxia? In asphyxia the right heart and great veins become engorged with blood. The blood in the cava must, therefore, be dammed back and if the adrenal goes on steadily secreting epinephrin the concentration in the upper segment of the cava must increase without any change having occurred in the rate of output. If the animal, in struggling or gasping, markedly increases the intraabdominal pressure the cava might be more or less obstructed by flattening of its walls upon the catheter, especially above its upper end.

There are still other ways in which the blood flow may be altered through effects produced upon the heart by the factors studied by Cannon. Yet in none of his papers has he indicated that there is any necessity to take into account possible changes in the rate of blood flow. It is the same with the question whether massage of the glands, particularly of the right adrenal, may not be produced by the catheter during the voluntary movements executed by the animal when frightened or the movements of the diaphragm in asphyxia. The right adrenal lies close against the cava, separated from the catheter by little more than the thin wall of the vein, and it is well known that massage causes liberation of epinephrin from the adrenals. All these possibilities ought to be controlled.

In referring to our negative results with the catheter method Cannon states, what could not be concluded from anything in his previous papers, that "the method is difficult and exacting, and that not until after some experience with it did it begin to yield us positive results." He rebuts our suggestion that some of his positive results might be due to a fortunate location of the eye of the catheter with reference to the orifices of the adrenal veins by saying "that it seems to have been made with disregard for the care exercised in making control observations under precisely the same conditions before and after stimulation." This is beside the point. There is no particular difficulty about inserting a fine catheter into the cava, so that in successive observations a string tied on the catheter is at the same level of the femoral vein, while the eye of the catheter is judged to be above the level of the adrenal veins. It is easy to turn the catheter so that a mark on its circumference always occupies the same position. But the exact relation of the eye of the catheter to the orifices of the adrenal veins or to the orifice of one of the veins, e.g., the right, since they do not enter the cava at the same level, can never be known till the abdomen is opened, if then, nor can it be assured that this relation will always remain precisely the same during such movements as the animal may make, even when securely tied down, during excitement caused by a barking dog or during stimulation of sensory nerves or asphyxia.

The few curves published by Cannon, are not at all convincing. In all the work with the catheter method he has contented himself with one or two comparative tests of the bloods. We have laid stress on the necessity of repeated comparison. His method of emptying the cylinder by sucking out one liquid

before the application of the next is also liable to introduce error, as we have previously pointed out (18) and illustrated by curves. Not only may the tracing be deformed at the critical moment, but the strip or segment is suddenly exposed and then suddenly immersed in liquid. He refers to a paper by three young medical men (17) as having confirmed his results on emotional excitement. But this paper contains not a single tracing or protocol, not one estimation of the epinephrin concentration in the blood or of the concentration which it would have been possible for the segments to detect. There is internal evidence that any reaction obtained could not have been due to epinephrin. For example, they state that when blood collected from the cava above the level of the adrenals, during the action of factors which increase epinephrin output, was allowed to stand for 25 minutes, it no longer caused inhibition of the rabbit's intestine segment, "and epinephrin is the only oxidizable substance in the body that produces inhibition of the intestine which can be oxidized in 25 minutes." Now, a sample of blood, known to contain epinephrin in amount sufficient to give a decided inhibition of the intestine, does not become ineffective merely by standing for 25 minutes. At one place it is stated that morphia prevents the epinephrin reaction from being given by the cava blood under the influence of Witte's peptone, and in another place that morphia distinctly diminishes the normal output of epinephrin, as tested in blood taken directly from the adrenal vein. In still another place the statement occurs that "substances with which we have experimented consisted of the toxins of gonococci, of streptococci, of staphylococci, of colon bacilli, of tetanus bacilli, of diphtheria, foreign proteids, indol and skatol, leucin, creatin, feces extract, strychnin, *morphia*, carbolic acid, Witte's peptone, sheep serum, ox serum, kitten serum. All of these have seemed to cause adrenal activation, and from their use 61 out of 66 consistent experiments can be reported Narcotics and anesthetics caused no increased output of adrenalin The various agents which caused increased epinephrin output, or which diminished it, are either neutral as to motion and fever or diminish them. There was no epinephrin reaction in any animal (subjected to intense emotion, rage and fear) in which bilateral division of the splanchnic nerves existed Of great significance is the fact that all agents that produced an increase of adrenalin output cause also a hyperchromatism of the brain followed by exhaustion. It mattered not whether this stimulating agent was a physical exertion in running, in fighting or in convulsions; or whether it was the emotion of fear, or of anger, or a reaction to anaphylaxis, to toxins, to indol, skatol, foreign proteids, or strychnin, in every case hyperchromatism and increase of adrenalin output went hand in hand." All this without tracings, without any sample of the great mass of experimental details which would be necessary to establish so many conclusions. If it is to be a matter of taking anybody's word for a physiological result, with all due respect to these writers, whom we know to be very competent men in their own subjects, we should much sooner take the word of an accomplished physiologist like Doctor Cannon himself.

In Cannon's paper on emotional stimulation the first figure shows that adrenal vein blood, obtained under ether, when substituted for inactive blood caused relaxation of the intestine strips, whereas blood from the renal vein did not give this effect. The most which could be deduced from this is that adrenal vein blood has a demonstrable content of epinephrin, a proposition which is generally

accepted. The second figure shows that when so-called excited blood (i.e., blood removed through the catheter from above the adrenal level during excitement) was substituted for Ringer's solution, the tone of the intestine strip was markedly increased. It remained increased for about $4\frac{1}{2}$ minutes, and then the strip relaxed and the beats disappeared. Cannon interprets this extremely belated relaxation as an epinephrin reaction. This is contrary to all our experience. An epinephrin relaxation is produced immediately or not at all. A relaxation occurring 4 or 5 minutes after the application of a blood cannot be accepted as a positive reaction for epinephrin. A second application of the excited blood, this time replacing so-called "quiet" blood caused a prompt relaxation. The only conclusion which can be drawn from this figure is that two inconsistent observations with a given sample of blood on such an object as an intestine strip or segment cannot be accepted as evidence that the blood from the inferior cava, drawn during excitement, gave a positive epinephrin reaction. If an undoubted positive reaction had been obtained, this, as already pointed out, would yield no information as to whether the rate of output of epinephrin had been increased by the excitement or not.

The third figure professes to demonstrate that with the prolongation of excitement the effect on the epinephrin secretion goes on increasing. This is done by comparing a record of the strip in defibrinated blood drawn from the cava after 11 minutes excitement with a record in the *serum* of blood obtained after 15 minutes of excitement. In the defibrinated blood the strip retains the increase of tone caused by substituting the blood for Ringer's solution, for about 2 minutes, and then begins to relax. In the serum the strip relaxes more promptly. If the two samples of blood had the same concentration of epinephrin, the serum would necessarily give a stronger reaction than the blood since practically all the epinephrin is in the serum (19). After the initial increase of tone has lasted 2 or 3 minutes some relaxation occurs also with "quiet" serum, and the beats become slower. No data are given by which it would be possible to judge how great the difference in epinephrin concentration in the different samples was.

The fourth figure is intended to show that after removal of the adrenal glands, blood from the cava of an excited animal above the adrenal level gave no inhibition of the intestine strip when substituted for Ringer's solution. As there is no question that epinephrin exists in the upper cava blood, so long as the adrenals are intact, and must disappear when the adrenals are excised, nobody will dispute that a test object, if sensitive enough, will give a positive reaction in the first case and not in the second. In the legend of this figure, it is stated that "the strip later proved sensitive to adrenalin in blood in the ratio 1:1,000,000," and in the fifth figure tracings are given showing the inhibition produced by blood with 1:1,000,000, 1:2,000,000 and 1:3,000,000 adrenalin when substituted for "quiet" blood. The 1:3,000,000 adrenalin blood gives a reaction much smaller, and the 1:2,000,000 adrenalin blood a reaction probably smaller than those figured by Cannon as given by "excited" blood. He does not state specifically that these curves were obtained from strips used to test any of the "excited" bloods whose records are reproduced, and, as has been pointed out, it is unfortunate that he did not assay the concentrations of epinephrin which he believed these bloods to contain. But there would seem to be no point in reproducing tracings showing the inhibition of intestine strips given by such concentrations

of added adrenalin, unless they had a bearing upon the magnitude of the reactions given by the cava bloods. Redfield (20) who worked with Cannon's method, and partly under his direction, has stated that he succeeded in modifying the method so as to render it capable of detecting 1: 10,000,000 adrenalin. Redfield obtained such variable results in experiments, in which he endeavored to detect epinephrin in the blood (of the horned toad) during emotional excitement by means of the intestine reaction, that he says "no weight can be placed upon the experiments."

It seems pretty clear that in Cannon's hands the method was not very sensitive, and that such inhibitions as he figures, if due to epinephrin, must have corresponded to epinephrin concentrations in the "excited" cava blood of the order of magnitude of 1:1,000,000 or 1:2,000,000. Now, these concentrations are double the average concentrations found in adrenal vein blood, with the average normal arterial pressure under the conditions of our experiments, and would represent 100 to 200 times our average normal output. Cannon in his last paper (4) has stated that with sciatic stimulation he can increase the output to 5 to 25 times our "normal" output. But that apparently is nothing to what can be obtained when a cat is frightened by a dog. For us, such concentrations in the cava blood (corresponding perhaps to 1: 10,000 in the adrenal vein blood) are so improbable that we are unable to accept them on such evidence as Cannon has furnished. If anything like such concentrations of epinephrin could exist in the cava blood with ordinary rates of blood flow, it would be impossible for us to miss them with the method of assay which we employ.

In the sixth figure a tracing is given, showing that "active" blood applied to a strip caused relaxation after 2 minutes. The same blood, after oxygen was bubbled through it for 3 hours, when applied to a *fresh* strip is stated to have failed to cause relaxation. If the reactivity of the new strip was the same as that of the other (which was not controlled, but which must always be controlled if reliable results are to be obtained with such test objects) this would indicate that an oxidizable substance (epinephrin) was responsible for the original inhibition.

Cannon cites Redfield (20) as having "reported that in the horned toad nervous excitement causes a contraction of the melanophores in the denervated skin, a reaction which does not occur after the removal of the adrenal glands." He adduces this as a proof that in this animal emotional excitement increases the epinephrin output. As a matter of fact, Redfield found that in the great majority of his experiments removal of the adrenals "does not check the contraction of the melanophores." Out of a large number of animals he only succeeded in finding two in which after adrenalectomy (involving the opening of the body cavity, removal of the gonads and a portion of the genital ducts and tying off the posterior cava) contraction of the melanophore pigment could not be obtained "when the mouth was stimulated by a weak faradic current." There is no proof that in these two exceptional cases it was the loss of the epinephrin output of the adrenals and not some other factors, such as the general deterioration of the animal caused by the operation, which was responsible for the negative result. However, his experiments with denervated skin and temporary ligation of the blood supply of portions of skin suggest strongly that epinephrin given off from the adrenals is a factor in contraction of the pigment. But we do not

find any proof that the effect is due to an increased epinephrin output. An experiment is quoted to show that blood from an excited horned toad causes a contraction of the melanophore pigment of an unexcited animal in the neighborhood of the point of injection, whereas blood obtained from an animal after destruction of the portion of the thoracic cord, whose integrity is essential to the production of contraction of the melanophore pigment through noxious stimuli, has no such effect. All that this experiment could indicate would be that the effect of the blood from the animal excited with intact cord was due to epinephrin. The experiment throws no light upon the question whether excitement can increase the epinephrin output. For if the injury to the thoracic cord prevents the contraction of the melanophore pigment associated with stimulation, by interfering with the ordinary output of epinephrin, the blood of the excited animal would necessarily contain more epinephrin than that of the control, without any increase in the rate of output having occurred in the former. We have shown (21), (22) that in mammals the upper part of the thoracic cord is intimately related to epinephrin secretion. The possibility must be considered that in this animal, on account of slower circulation, less active metabolism, and perhaps lower temperature of the blood and tissues, a greater concentration of epinephrin may exist in the general blood than in mammals. The great concentration found by Abel and Macht (23) in the secretion of the so-called parotid gland of a tropical toad suggests that care may be necessary in applying results obtained in such animals to mammals. The above remarks are in no way intended as a criticism of Redfield's paper, which seems to us a most interesting and suggestive piece of work.

In Cannon's paper on the effects of asphyxia, hyperpnoea and sensory stimulation on adrenal secretion there are three figures. Rabbit's intestine segments were employed for the tests. The first figure shows a marked effect of disturbance of the curve by sucking out the liquid. If any weight can be laid on a curve of this kind, the "normal" vena cava blood (i.e., from above the adrenal level in the absence of asphyxia) probably caused some inhibition and the cava blood from the same level after asphyxia, a greater inhibition. But how is it possible to determine whether the difference in epinephrin concentration between the two cava specimens is great or small?

The second figure purports to demonstrate the failure of hyperpnoea to increase adrenal secretion. "The chest was opened and the chest walls held apart while the lungs were inflated in rapid repetition by means of bellows. The air forced into the lungs was permitted to escape quickly through an opening in the trachea." The curve shows that when vena cava blood taken from above the adrenals with ordinary breathing is substituted for Ringer's solution, there is some increase of tone, but the beats continue. When vena cava blood drawn from above the adrenals after the application of hyperpnoea is substituted for the previous sample of cava blood no notable change occurs in the tracing. The conclusion is "that hyperpnoea, to a degree resulting in acapnia is not attended by increased adrenal secretion." This seems to us extremely probable, but what the tracing indicates is simply that no noteworthy change has occurred in the concentration of epinephrin in the cava blood, within the limits of sensitiveness of the segment. It is obvious that the conditions which in the asphyxia experiments may lead to slowing of the blood flow in the cava are absent here.

The chest being widely opened, changes in the intrathoracic pressure and in the movements of the diaphragm are eliminated. The situation as regards vasomotor and cardiac changes, especially splanchnic vasoconstriction, is likewise quite different. Since Cannon has here performed a considerable operation in an anesthetized animal, and since, according to him, the trauma and anesthesia in our experiments increase the "ordinary" output of epinephrin so much that it may be impossible for asphyxia or sensory stimulation to cause any further increase, the question would seem pertinent why he obtains in this experiment a totally negative reaction for epinephrin in the cava blood taken above the adrenal level. Our explanation is that the small adrenal output is too much diluted by indifferent blood in the cava to be easily detected.

The third figure in this paper purports to show that sensory stimulation increases adrenal secretion. What it does show is that when cava blood from above the adrenal level, obtained during sciatic stimulation, is substituted for cava blood obtained without sciatic stimulation, the writing point descends abruptly. A large part of the descent appears to be due simply to the fact that when the latter blood was sucked out of the cylinder the weight of the segment, coming on the lever, raised the point considerably. When the cylinder was again filled up with blood, the weight of the segment was nearly neutralized and the writing point necessarily descended. The true effect of the inhibition of the segment is, therefore, considerably exaggerated. All that can possibly be deduced from the tracing is that the epinephrin concentration in the sample collected during sciatic stimulation was greater than in the other. No assay of the concentration was made and, therefore, it is impossible to know what the difference was. It might have been a very moderate difference or a very great one. Inspection of such a curve can yield no information as to this. Also, it is not stated how many times the result figured was confirmed on the same samples of blood.

In his paper on the effect of nicotine on adrenal secretion Cannon has published three figures. The first shows that cava blood from above the adrenal level, secured before nicotine injection, when substituted for Ringer's solution caused an increase of tone of an intestine strip. When cava blood from the same level, obtained after injection of nicotine, was substituted for the other cava blood, there was a prompt and large diminution of tone. The conclusion is drawn that the adrenal secretion was increased by the nicotine. The most, however, which can be deduced from the tracing is that the concentration of epinephrin in the second specimen is greater than that in the first. If the blood flow is slowed, as would be the case after the doses of nicotine employed, an increased epinephrin concentration would be consistent, not only with an unchanged, but even with a diminished output. We have already pointed out (24) how completely Cannon, owing to the faulty method employed, has missed the details of the nicotine action on the epinephrin output, the most outstanding and most durable effect being a depression of the output.

It is stated in the text that the several specimens of blood were tested for their content of epinephrin. But not one tracing illustrating the assay is reproduced, nor is a single concentration given, although the statement is made that "the characteristic inhibition of the rhythmic contractions of the muscle, even when it has been for some time removed from the body, occurs at a dilution of adrenalin 1:2,000,000 in defibrinated blood." If it is implied that this is the order of

magnitude of the concentrations which cause inhibitions like that displayed in the figure under discussion, we can only repeat that it is in the highest degree improbable that such concentrations can be obtained in a fair sample of the cava blood above the adrenal level, unless there is an extreme slowing of the cava flow. Our own work on nicotine shows that at the time Cannon collected his samples the initial brief stimulating action of nicotine on the epinephrin output must have long since passed into the stage of depression. The effect of the first cava sample when substituted for Ringer's solution cannot, of course, be compared with that of the second cava sample when substituted for the first. To assay the two specimens they should be caused separately to displace the same sample of indifferent blood free from epinephrin. The displacement of one sample of epinephrin-containing blood by another sample of epinephrin-containing blood is not usually satisfactory for purposes of assay. For *a*, the first sample has already produced an epinephrin effect upon the test object which may render the inhibition, caused by the second sample when it displaces the first, different from what it would otherwise be. *b*, The epinephrin content of the first sample in contact with the test object, especially if left for a considerable time in contact with it, is diminished, so that the content of the second sample when now caused to displace the first may be exaggerated.

The second figure in Cannon's nicotine paper shows that the quantity of nicotine employed could not have accounted for the inhibition of the intestine strip produced by the cava blood after nicotine injection. This is unquestionably correct. The third figure demonstrates that cava blood obtained from an animal after removal of the adrenals and after administration of nicotine, does not cause an inhibition of the intestine strip. This is no doubt perfectly true, but it simply indicates that in the absence of the adrenals no epinephrin detectable by the method employed exists in the cava blood. It would be much more to the purpose to state what were the concentrations of epinephrin in the cava blood and the rates of cava blood flow before and after injection of nicotine. But the reader searches in vain for this essential information. That the intestine strip was inhibited by the addition of one drop of adrenalin 1:1000 to a cylinder containing 2.5 cc. of blood (which would make a concentration of about 1:35,000 or 1:40,000) is no doubt correct. But what possible relation has a huge concentration like this to anything which can occur in the cava blood?

SUMMARY

1. The acceleration of the heart induced by asphyxia after division of the vagi and excision of the stellate ganglia cannot be taken as an index of increased epinephrin output from the adrenals, as assumed by Cannon. For it may be obtained and may not be diminished in amount after the adrenal veins have been ligated, after the adrenals have been completely isolated from the circulation, and after the adrenals have been excised.

2. Toward the end of an experiment, after repeated periods of asphyxia and when the general condition of the animal, including the

circulation, has deteriorated the reaction is apt to become much less marked or to fail altogether. An operation like adrenalectomy, when it accelerates the deterioration of the animal and of the heart by lowering the blood pressure decidedly, may sometimes seem to be responsible for the failure of the asphyxial acceleration. But there is evidence that this is not due to any specific effect of the adrenalectomy (loss of epinephrin output) but to the general effect of the operation. When asphyxia fails to cause a decided acceleration it is often seen that some slowing has occurred during the asphyxial period, succeeded by a quickening of the beat on resumption of respiration. It is clear that it will depend upon the state of the heart whether this subsequent acceleration will bring the heart back to, or nearly to, its original rate or cause it to pass considerably beyond the original rate. Whether an acceleration of some magnitude, as compared with the rate before asphyxia, can actually be counted on the trace is then more or less of an accident, depending upon whether the deteriorated heart comes through the period of asphyxia in such a condition as to permit it to attain a rate considerably in excess of the initial rate.

3. There is no good evidence that asphyxia causes an augmentation of the rate of epinephrin output by a direct action upon the cells of the adrenal medulla.

4. The acceleration caused by asphyxia, like the acceleration caused by stimulation of the central end of the sciatic, is not interfered with by opening the abdomen. There is no evidence that the operation necessary for collecting adrenal vein blood for direct assay of its epinephrin content would render it impossible to detect an increase in the epinephrin output, if this were caused by asphyxia or stimulation of sensory nerves.

5. No real evidence has been adduced that the epinephrin output, measured by those observers who have adopted the fundamentally correct method of collecting the blood and assaying it on suitable test objects, is an output artificially increased by anesthesia and trauma. On the contrary, the remarkably narrow range of the output in different animals, under different anesthetics and with the different operations strongly suggests that it is a physiological output already going on, not initiated and probably not much modified, but merely unveiled by the experimental procedures necessary for its measurement.

6. As a method of estimating changes in the rate of output of epinephrin from the adrenals, the catheter method is defective in principle. For at best all that could be measured by it would be changes in the

epinephrin concentration in the blood of the inferior cava above the level of the adrenals. Changes in the rate of flow of the blood are not taken account of. The quantity of epinephrin passing along the inferior cava to the heart per unit of time cannot be estimated, nor the changes, if any, produced in this quantity by the conditions studied. We have pointed out that in the cases in which Cannon claims to have obtained evidence of an increased output of epinephrin, all he can possibly have observed is an increased concentration in the cava blood, and that a slowing of the cava flow would cause an increase in the concentration, if no change whatever had occurred in the rate of output. We have suggested certain factors, associated with all the conditions studied by him, which might cause such changes in the cava flow as would tend to increase the epinephrin concentration, even in the absence of an increased rate of output. However, the complete lack of assays of the concentrations of epinephrin supposed to have been present, and of estimates of the concentration which could have been detected by each segment or strip employed, renders it impossible to determine to what extent positive reactions, when obtained, were actually due to epinephrin. It cannot be assumed that by sucking blood from a catheter opening into the cava immediately below the diaphragm, a region where the blood flow is necessarily less steady than anywhere else, owing to the influence of changes in the intrathoracic pressure, the same aliquot part of adrenal blood will be drawn off in successive samples without regard to the gross effects upon the respiration produced by such conditions, e.g., as asphyxia. Such mechanical changes may alter the proportion of adrenal blood to the much greater quantity of blood from other sources drawn off through the catheter. Our experience with the catheter method fully justifies the criticism of Richards and Wood (25) that the method is "highly faulty in that the blood from the suprarenals is diluted with that from all of the structures whose veins enter the cava below the suprarenals."

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